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# PLANT PHYSIOLOGY

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## F. A. F. C. WENT[1] (1863–1935)[2]

GEORGE J. PEIRCE

(WITH PLATE II)

FRIEDRICH AUGUST FERDINAND CHRISTIAN WENT was born in Amsterdam, Holland, on June 18, 1863, and died in Wassenaar, July 24, 1935. Between these two dates was a human life, composed of those elements common to all of us, and of experiences and achievements common to no other. To recognize this is one thing; to describe it is another.

WENT was the eldest son of JOHANNES and JOHANNA WENT. His father, JOHANNES WENT, prepared for the ministry, but when he received the degree in theology there were more candidates than pastorates, and he became a stockbroker. The son passed through the Amsterdam schools and in 1880 matriculated in the University of Amsterdam as a student of Botany. One can recognize in the manner of his thinking, the course of his teaching, and the products of his pen in after life, the impress of his teachers during the years of academic preparation. They were VAN 'T HOFF, VAN DER WAALS, MAX WEBER, OUDEMANS, and DE VRIES. Sound training, real facts, and with them the example and the inspiration to explore, to conjecture, and to experiment. The Doctor's degree was conferred upon him in 1886. In his dissertation on the earliest stages of vacuoles he reported his observation that vacuoles arose, in the plants which he studied, only by division of preexisting vacuoles, and not *de novo*. The bearing of this on cytomorphology I need not point out. For some years he continued his studies of vacuoles in land plants, and in marine plants (in Naples, 1888), in the meantime studying in STRASBURGER'S laboratory in Bonn, and with VAN TIEGHEM in Paris. By these visits to other countries he improved his lin-

[1] The accompanying portrait of WENT is a reproduction of a pastel from life by H. J. HAVERMAN. The original is in the collection of portraits of Eminent Naturalists, Stanford University.

[2] Acquaintance has been supplemented by dates and data generously furnished by Professor F. W. WENT, of the California Institute of Technology in Pasadena, to whom I express my appreciation and gratitude.

guistic accomplishments, thus facilitating the expression of his generous spirit and promoting that international cooperation which is the recognition of the unity of science, as of the fine arts.

Like many other Netherlands botanists, WENT spent several years in Java, the first few months in the famous Botanical Garden in Buitenzorg, the later years as Director of the Agricultural Experiment Station of West Java. Whether because of TREUB'S cosmopolitan character and interests, or of the liberality of the Netherlands Government, at all events the Garden, under TREUB'S direction, became the Mecca of botanists the world over. Many visited this tropical botanical garden, seeing what plants can do when supplied with warmth, water, minerals, and solar energy in abundance. In such an environment the abundance intensifies and makes very real the struggle for existence. Pests and parasites thrive no less than their hosts. Hence, when WENT was appointed Director of the Sugar Cane Experiment Station, he undertook the study of sugar cane diseases. His study quickly showed the need of knowing the normal physiology of a plant in order successfully to combat its diseases. Another result was the rescue of the Javanese sugar industry then threatened with destruction by the so-called "sereh" disease. WENT'S success in this respect no doubt reinforced his contention that the colonial agricultural experiment stations should not be directed exclusively from any home office, but that they should be autonomous, free to study, and to meet local needs.

These revealing years in the tropics, following a few years of filtering his knowledge by what we should call high school teaching, led to WENT'S appointment in 1896 as Professor of Botany in the University of Utrecht, a position which he filled, and filled increasingly, until his retirement in 1934.

The members of this Society most naturally associate WENT'S name with studies in plant physiology; but that his knowledge and his interests were not limited to physiology, or to physiology as applied to industry, is proved by his contributions to the development of science in many fields, and of society. In support of this statement may I sketch his history after returning to Holland.

First as to academic position. On his return from the tropics he succeeded to an honorable post in a distinguished university and an assured position in an established social system. He used these not to rest upon but to build upon. Youth, temperament, and talent contributed to this, but above all was the interest in his subject, the study of living things, especially of plants. After his study of vacuoles in the cells of algae, land plants were his chief concern.

In accordance with the general system in the universities on the continent of Europe, there were students or there were not, in a given subject

in a given place, depending upon whether at that time there was a fertile representative of that subject on the staff. There were, as there are now, centers of study with a man or a group of men as a magnet. Young people were not drawn, as in this country, by the glamor of a historic name—Harvard, Yale, Princeton—but they went to men like STRASBURGER, PFEFFER, VAN TIEGHEM. Utrecht, Leiden, and Amsterdam were intellectual centers, but only because of living leaders in physiology, medicine, and botany, not because of age or geography. So Utrecht, with its very few students of biology, except those going into medicine, was not thought of by young botanists in 1896 as a place to go to and to work in, but it became so. The reason for this was WENT. The change came only slowly, however. For years WENT had no associates in botany of any but subordinate age and position, although ENGELMANN (soon followed by ZWAARDEMAKER) in physiology and PEKELHARING in anatomy were there, and at first whatever botany was taught at Utrecht was given by him. Nearly twenty years later he was relieved by the appointment of PULLE, in 1914, as Professor of Taxonomy and Plant Geography. I emphasize the title of this professorship because of the information it gives as to the thinking of this man, whose early work was cytological and morphological, on one of the plant families (Podostemonaceae). He was concerned with *living* things. In 1917 Miss JOHANNA WESTERDIJK was appointed Professor of Phytopathology in the University of Utrecht and in 1928 or 1929 in the University of Amsterdam as well. In 1930 HONING became Professor of Genetics in Utrecht also, as he already was in WAGENINGEN. In 1926 DE BUSSY, Director of the Museum of the Colonial Institute, became the Professor of Tropical Botany. These appointments illustrate that spirit of scientific cooperation of which WENT was a leading advocate and example.

The record of doctorates in botany at Utrecht in the successive decades of WENT's term as Professor of Botany and head of the botanical establishments in the University is significant.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Between | 1896 | and | 1905 | there | were | 2 | doctorates | in | Botany |
| " | 1906 | " | 1915 | " | " | 14 | " | " | " |
| " | 1916 | " | 1925 | " | " | 17 | " | " | " |
| " | 1926 | " | 1934 | " | " | 29 | " | " | " |

These figures must be considered in connection with scientific and economic conditions elsewhere. During the Great War the German universities, to which foreigners had continued to resort, although in decreasing numbers, were closed to the outside world. In 1920 PFEFFER died, a victim of the war. CZAPEK, his successor, died before he could establish himself in PFEFFER's stead. There remained no one in the German-speaking coun-

tries who had won eminence, either by his own work or that of his students, in the Pfefferian type of investigation of plant physiological problems. But WENT naturally became the successor, although he never studied with PFEFFER. So there began that stream of plant physiological studies of respiration, growth, and response, devised and directed by WENT, some of them reported by WENT to the Netherlands Royal Academy, all offered as dissertations for the Doctor's degree, and forming that contribution to botanical science which made the Utrecht laboratory famous.

WENT's contribution to plant physiology, however, did not consist merely in continuing the kind of work associated with PFEFFER and his laboratory, nor in making as nearly quantitative as possible what had until then been mainly qualitative; but reflecting on the phenomena of growth and wondering about its cause and control, WENT began those investigations continued by his son, by DOLK, and by others, which demonstrate the existence and the importance of SACHS's postulated "Wuchsstoff." The conception of hormones is now common, and some day it may become clear! At all events the experiments devised by father and son, and made by the son in the father's laboratory, have demonstrated the existence of one or more "Wuchshormone" or auxins, and have definitely introduced into plant physiology the general conception of regulators or coördinators—instigators, inhibitors, etc. The unmaterial stimuli, light, heat, and gravity, affect the distribution of the growth hormones which, if they move from cell to cell or from organ to organ, become "messengers." In this line of investigation one recognizes the bridge which connects the "general" physiology of today with the "old fashioned" physiology of the founders; so that, with hormones, inhibitors, ions, and positive and negative charges, our vocabularies are complete, whatever our knowledge of the living organism may be.

A further contribution of WENT's to plant physiology consisted in the training and inspiration which he gave to that group of young and vigorous scholars who are continuing and extending his methods, his interests, and his ideals. The inspiration which he received he broadened, deepened, and transmitted. It is thus that a teacher prolongs his life and usefulness.

By travel and by attendance at national and international gatherings of scientific men he extended mutual acquaintance beyond the boundaries of his own country and of his own subject. Not to mention meetings nearer home, he attended, as Government Representative, the International Rubber Congress in Java in 1914; he was at Ithaca, New York, in 1926, attending the international meeting of those interested in the plant sciences; he traveled westward across the North American continent, visiting on the way; and across the Pacific to the Pan-Pacific Science Congress in Japan; and again he was present at the Pan-Pacific Science Congress in Java in 1929, visiting Ceylon *en route*.

On his trip to South America in 1901 he met CATHARINA TOUCHENS, daughter of WARMOLT TOUCHENS, Governor of Dutch Guiana, in Surinam; and they married in 1902. They had five children, namely, FRITS WARMOLT WENT, Professor of Plant Physiology, Pasadena; JOHANNA CATHARINA WENT, Doctor of Phytopathology; JAN JACOBUS WENT, Doctor of Physics, Research Assistant in the laboratories of Philips Inc., Eindhoven; FROUKJE MARIE WENT; LODEWYK NICOLAAS WENT; they, with their mother, survive him.

WENT was a recognized leader in national and international, as well as in local academic matters. He was chairman of the Division of the Physical Sciences in the Netherlands Royal Academy for ten years, 1921–1931; he was to be the President of the International Botanical Congress of 1935, and was directly concerned in all of the preparations for the remarkably pleasant and profitable gathering which took place nearly two months after his death. Throughout the meetings and excursions of the Congress he was spoken of, his loss deplored, his worth acknowledged. The gracious effectiveness with which he would have conducted the meetings of the Congress was the example followed by his substitute, SCHOUTE.

WENT's eminence as a scientific man was recognized by the universities (Padua and Cambridge) which conferred honorary degrees upon him, and by the twenty academies and societies which elected him to membership, foreign, honorary, or corresponding.

The death of WENT this last year brings to a close one of the fullest and most significant lives ever dedicated to science.

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# PHOTOPERIODIC RESPONSES OF CERTAIN GREENHOUSE ANNUALS AS INFLUENCED BY INTENSITY AND WAVELENGTH OF ARTIFICIAL LIGHT USED TO LENGTHEN THE DAYLIGHT PERIOD

R. B. WITHROW AND H. M. BENEDICT
(WITH NINE FIGURES)

## Introduction

Two types of experimental method have been employed by investigators in the field of photoperiodism. In the one case the experiments have been conducted during the long days of summer and the natural daylength used for the long day treatment, with the short days produced by moving the plants into dark chambers after a certain number of light hours. In the second type of experiments the investigations have been carried on during the short days of winter and the natural daylength used for the short day period, the long days being produced by the addition of artificial radiation.

It might be questioned as to whether the results of the two experimental methods are comparable and whether both involve the same group of physiological processes, since the conditions of radiation for the two differ so widely. That those conditions used in the greenhouse during the winter do not simulate the conditions under which the plant would respond during its natural growing season must be recognized. This is especially true in regard to light intensity and spectral energy distribution.

In the first paper published by GARNER and ALLARD (**1**) on the effect of the photoperiod on the flowering of plants, it was shown that typically long day plants became reproductive and short day plants assumed the vegetative state when they were subjected to naturally short winter days artificially lengthened with relatively low intensity incandescent lamp radiation. TINCKER (**14**) obtained similar results with several long and short day plants when he used 5 foot candles of visible radiation to supplement the natural daylight period. TINCKER (**15**) conducted experiments during the summer in which he produced the short day condition by complete darkening after a definite number of light hours. The results which he secured as to morphological characteristics are comparable in many respects with those obtained by RAMALEY (**10, 11**), who used incandescent lamps to lengthen the days of the winter season. LAURIE and POESCH (**8**) add further evidence with regard to flowering responses by means of artificial light and shading experiments. It thus appears that similar photoperiodic effects may be obtained by either type of experimental method, and that in both cases the differences in response are largely due to differences in the duration of the lighted period.

GILBERT (**5, 6**), THOMPSON (**12, 13**), WERNER (**16**), and PURVIS (**9**) have shown that temperature may exert a marked influence on the flowering habit, formation of storage organs, and general growth habits of plants, and that the factor of temperature must be taken into careful consideration in all photoperiodic studies. The temperature at which the plants are grown should be noted and carefully controlled. Furthermore, attention should be given to changes in temperature of the plant tissues, due to the heating effect of the artificial radiation. To obtain high intensities of the order of hundreds of foot candles of visible radiation with an incandescent lamp, the total radiation of the unfiltered lamp may be high enough to produce appreciable heating of the leaves above the temperature of the surrounding air, since the total energy emitted is high owing to the large percentage of infra-red produced as compared with the visible. The possibility of heating effect has been minimized in most phases of the present investigation.

When artificial radiation is used to lengthen the short days of winter, several radiation variables must be taken into consideration. The two most important ones are the intensity of artificial radiation required to bring about the photoperiodic response and the influence of the various spectral regions on this response.

On the basis of these considerations, preliminary experiments were begun at the Purdue University Agricultural Experiment Station in the winter seasons of 1930 and 1931 on the photoperiodic responses of several greenhouse annuals, when the normally short winter days were lengthened with various intensities and wavelength bands of incandescent lamp radiation.

In the first experiments on intensity, reported by GREENE *et al.* (**4**), pansy (*Viola tricolor*), China aster (*Callistephus chinensis*), and calendula (*Calendula officinalis*) were subjected to incandescent lamp intensities of from 8 to 125 foot candles for five hours each night. In the case of pansy, little additional increase in flower production was exhibited under intensities beyond 20 foot candles. Aster came into flower under all intensities of artificial light at approximately the same time. The controls, which received only the natural radiation in the greenhouse, had not flowered by spring. Calendula responded with increased stem length and the flowering stems in the lighted plots were all much longer than those in the controls.

Thus the responses of these three plants to intensities ranging from 8 to 125 foot candles indicated that other factors than light become limiting at relatively low light intensities.

In later experiments reported in 1933 (**17**), pansy, aster, and stock (*Matthiola incana*) were subjected to intensities varying from 1½ to 110 foot candles. Similar results again were obtained in which little additional increase in response was secured with intensities above a relatively low value of a few foot candles.

In 1933 (**18**), the results of experiments on the flowering of pansy, aster, and stock to incandescent lamp radiation filtered by a series of various dyed cellophane films were reported. The wavebands employed were red (1000–640 mμ), yellow-green (620–500 mμ), and blue-violet (500–400 mμ). The energies in the various bands were sufficiently balanced that the effects secured could hardly be attributed to differences of intensity.

The results showed that the red end of the visible spectrum produced the most marked photoperiodic responses in increased and earlier flowering in pansy and aster and increased stem length in stock and aster. The yellow-green region had the least effect, with the plants behaving much as did those in the controls. The blue-violet band seemed to produce a somewhat depressing effect on growth in some cases, although earlier flowering was secured with aster. In general, the filtered red radiation and the unfiltered Mazda lamp radiation produced the same type of response.

## Experimental procedure

The present investigation is a continuation of the same type of work conducted in the previous experiments. The plants received all of the daylight of the intensity and duration available in the greenhouse during the winter season. To this daylight were added various intensities of incandescent lamp radiation and also highly filtered radiations of relatively balanced energies. The artificial period was so controlled by means of time switches as to maintain a 21-hour day, the radiation being applied from three hours after sundown until dawn.

Each plot was separated from adjacent plots by black oilcloth curtains which were in place during the periods of artificial radiation. These curtains were removed during the day so that they interfered in no way with the normal solar radiation. In spite of this complete curtaining, it was impossible to prevent some scattered light from being reflected from the glass roof of the greenhouse and other overhead structures. The control plots were partially curtained overhead with black gabardine, in such a way as not to interfere too much with the normal movement of air currents and yet to reduce the scattered light intensity at the plants to less than 0.01 foot candle as measured with a Macbeth illuminometer. This intensity has been found to be the threshold value for a true check plot for a sensitive plant such as China aster. In several instances a value of 0.03 foot candle has been noted to produce a slight response with aster seedlings, inducing a tendency of the leaf petioles to turn upward.

Pansy seeds were sown August 10, 1933, in flats. The seedlings were transplanted into 2-inch pots and further shifted to 3-inch pots as they became older. On October 10 they were placed in their respective plots and the lights applied from this date until the close of the experiment, except

in the case of the controls or short day plants which received no additional artificial light. The pots were sunk into soil in raised benches in order to maintain a more uniform moisture supply. On November 28 all except 14 plants or thereabouts in each plot were harvested for the data presented in the height and weight tables. The remaining plants were benched with a spacing of $6 \times 8$ inches for the duration of the experiment. The plots were $4\frac{1}{2} \times 5$ feet.

Aster was handled in much the same manner as pansy, being seeded September 6, put on the bench for irradiation October 10, and a portion harvested and the remainder benched December 27 with a spacing of $8 \times 8$ inches.

Stock was similarly grown, being seeded September 14, set on the benches in pots for radiation October 10, and harvested and benched $3 \times 6$ inches as above on December 21.

The plants were all grown in a rich manure composted loam and given the usual commercial cultural treatment other than that just mentioned. The temperature was maintained at 60° F. during the day and 55° F. during the night.

### Light intensity series

Light intensities of 100, 10, 1, and 0.3 foot candles were used over pansy, stock, and aster. The intensities given are those maintained immediately under the lamps and midway between the soil surface and the average height of the plants. As the plants increased in height, the lights were elevated. At the edge of the plot, the intensity values were approximately 50 per cent. of the center values. Incandescent lamps in porcelain enameled bowl-type reflectors were used.

Leaf temperatures were measured by means of a thermocouple under the four intensity conditions. It was found that unfiltered incandescent lamps of wattages sufficiently high to produce 100 foot candles raised the leaf temperature above that of the surrounding air from 1° to 2° C. Light intensities of 10 foot candles or less did not make a measurable increase in leaf temperature. In order to eliminate this heating effect in the 100 foot candle plot, the radiation of the lamps was filtered through 5 cm. of distilled water in a glass-bottomed metal tank. This absorbed the infra-red of the wavelengths longer than 1400 mμ, it being tentatively assumed that infra-red radiation beyond this limit has no effect other than that of heating the plant tissues. Under these conditions, no increase in leaf temperature could be measured.

### Wavelength series

Considering the preliminary results with filtered radiation, the experimental work was continued with certain refinements, especially with regard

to filters. Eight plots were employed, including, beside the control, groups radiated with blue, green, yellow, red, and infra-red radiations.

A series of filters was developed using dyed gelatin films on glass. These were mounted below a filter cell cooled by means of tap water flowing through coils of copper tubing immersed in 10 cm. of distilled water, the latter absorbing the infra-red beyond 1400 mμ. Figure 1 shows the general

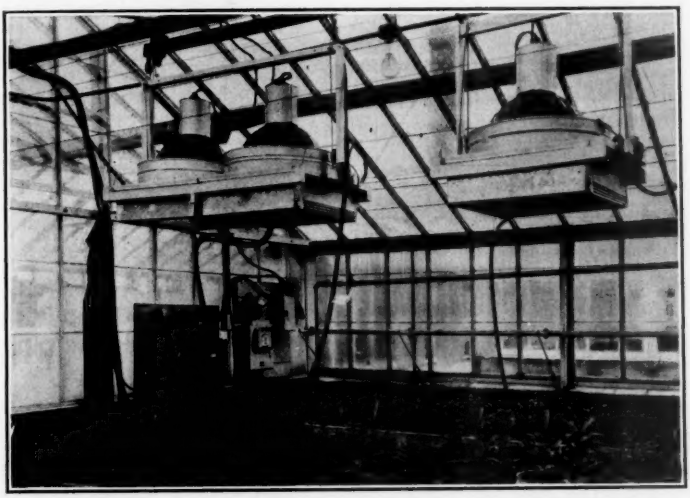

FIG. 1. Arrangement of water-cooled cells for filtering Mazda lamp radiation. Double cell is over the blue plot. In the background is shown the power panel containing clocks and magnetic switches for light control.

arrangement of these cells. The lamps were mounted in 1000-watt fluted bowl-type enameled reflectors which rested on the filter cell structure. The gelatin filters, on 20-inch square panes of window glass, were slid into grooves in the lower section of the cell.

The relative energy distributions of the radiation emitted by the filter systems for the lamps employed are plotted in figure 2. The curves were obtained by multiplying the transmittancy as given in table I by the relative energy of the incandescent lamp in the corresponding filter cell. The transmittancies were obtained with a Bausch and Lomb visual spectrophotometer. The energy distribution was calculated from the simplified table of HOLLADAY (7), assuming average color temperatures of 2980°K. for 1000-watt Mazda C, PS 52 lamps; 2920°K. for 500-watt Mazda C, PS 40 lamps; and 2810°K. for 200-watt Mazda C, PS 30 lamps.

The densities of the filters for the blue, green, and yellow were empirically

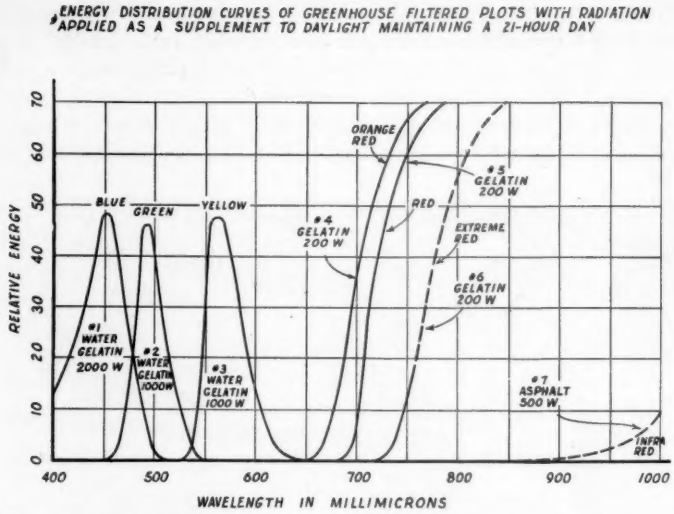


FIG. 2. Energy distribution curves of filter combinations used in the seven plots of filtered radiation.

varied until they produced the same maximum values of relative spectral energy when used with 1000-watt lamps. Since the transmission bands of the three filters were not of the same width, it was obviously not possible to keep the total energy and the peak values both equal for these three filters. It was considered more significant to maintain the peak values of the same magnitude rather than the total energy.

The blue, green, and yellow plots were designed to give some index as to the relative spectral sensitivity of the plants without seriously introducing the variables of intensity. The three red plots were planned to determine at what point in the red the photoperiodic response becomes of appreciable magnitude, and therefore only long wave pass filters without water cells were set up and little attempt was made to balance the energy relationship.

Since the red series of filters transmitted the near infra-red to varying unknown amounts, an infra-red plot was included to determine whether the infra-red beyond 760 mμ was capable of producing responses of any significance. The filter used in this plot transmitted 30 per cent. of the total energy of a 500-watt lamp, and it was just barely possible to see the filament of the lamp as a dull red coil.

The filters mentioned in table I were especially prepared for these investigations and the details of their preparation and application will be discussed in a subsequent paper.

## TABLE I

TRANSMISSION OF FILTERS USED IN WAVELENGTH SERIES

| Wavelength | Transmission | | | | | | |
|---|---|---|---|---|---|---|---|
| | Infra-red | Extreme red | Red | Orange-red | Yellow | Green | Blue |
| | Asphalt | Acid film green and pontamine fast red 8 BL | National brilliant green and pontamine fast red 8 BL | Carbic violet and pontamine fast red 8 BL | Orange G and cupric acetate | National brilliant green and cupric acetate | Carbic violet, Victoria pure blue BOR and cupric acetate |
| mμ | | % | % | % | % | % | % |
| 750 | Trans- | 15.0 | 72.0 | 75.0 | | | |
| 740 | mits | 10.0 | 65.0 | 72.0 | | | |
| 730 | infra- | 2.0 | 55.0 | 67.0 | | | |
| 720 | red | 0.0 | 38.0 | 63.0 | | | |
| 710 | 30% of | | 21.0 | 55.0 | | | |
| 700 | Mazda | | 9.0 | 50.0 | | | |
| 690 | lamp | | 0.5 | 44.0 | | | |
| 680 | energy | | 0.0 | 35.0 | | | |
| 670 | | | | 12.5 | | | |
| 660 | | | | 2.0 | | | |
| 650 | | | | 0.0 | 0.0 | | |
| 640 | | | | | 0.25 | | |
| 630 | | | | | 0.5 | | |
| 620 | | | | | 0.75 | | |
| 610 | | | | | 1.5 | | |
| 600 | | | | | 3.0 | | |
| 590 | | | | | 5.5 | | |
| 580 | | | | | 8.0 | | |
| 570 | | | | | 13.0 | | |
| 560 | | | | | 14.0 | | |
| 550 | | | | | 9.0 | 0.0 | |
| 540 | | | | | 1.0 | 0.5 | |
| 530 | | | | | 0.0 | 2.5 | |
| 520 | | | | | | 6.0 | |
| 510 | | | | | | 12.0 | 0.0 |
| 500 | | | | | | 20.0 | 0.5 |
| 490 | | | | | | 24.0 | 3.0 |
| 480 | | | | | | 16.0 | 5.0 |
| 470 | | | | | | 4.0 | 11.0 |
| 460 | | | | | | 0.25 | 17.0 |
| 450 | | | | | | 0.0 | 20.0 |
| 440 | | | | | | | 18.0 |
| 430 | | | | | | | 16.0 |
| 420 | | | | | | | 13.0 |
| 410 | | | | | | | 11.0 |
| Lamp wattage | 500 | 200 | 200 | 500 | 1000 | 1000 | 2 × 1000 |
| Cooling | Air | Air | Air | Air | Water | Water | Water |

Since organic dyes are relatively unstable to light, the filters were checked for change in transmission every two weeks and replaced with new ones as needed. It was found that even the most fugitive of the dyes used, Brilliant Green, required replacement not oftener than every three weeks. Pontamine Fast Red 8 BL, on the other hand, showed no signs of deterioration after a whole season of use for approximately 10 hours each night.

Since relatively low wattage lamps were used in the air-cooled filter cells, there was little trouble from overheating except with the asphalt infra-red filter, in which the glass panes had a tendency to crack in the early part of the experiment. This difficulty was largely overcome by using a thinner grade of window glass.

## Results

### Intensity series

Tables II, IV, and VI present the flowering data of pansy, stock, and aster. In the case of pansy and aster, the stem length given is that of the cut flower at the time of harvest. In general, the flowers were cut at intervals of several days throughout the blooming period. With stock, the stem length is in reality the height of the plant at the time approximately half of

TABLE II

Effect of intensity of light on flowering of pansy, variety Giant Swiss Blue, seeded August 10, 1933. Irradiation begun 8 weeks after seeding for 149 days from October 10, 1933, to March 8, 1934

| Light intensity foot candles | Number plants | Average number flowers per plant | Flower ratio | Average flower diameter | Average stem length | Total time to flower |
|---|---|---|---|---|---|---|
| | | | | *cm.* | *cm.* | *days* |
| 100 ..... | 14 | 32.3 | 6.6 | 4.9 | 13.4 | 106 |
| 10 ..... | 12 | 27.0 | 5.5 | 4.9 | 11.6 | 109 |
| 1 ..... | 14 | 12.2 | 2.5 | 5.5 | 11.9 | 114 |
| 0.3 ..... | 14 | 4.7 | 0.96 | 5.4 | 11.7 | 123 |
| Control* ..... | 14 | 4.9 | 1.0 | 5.8 | 10.6 | 178 |

* Average of two control plots.

the flowers on the main spike were in full bloom. The average number of florets of stock is the number of flowers which were in bud or open at the time of harvest. The flowering date for all the plots was taken as that date on which five flowers (or flowering spikes, as in the case of stock) had been harvested from the plot.

The data as presented in the tables and in the graph (fig. 3) show that

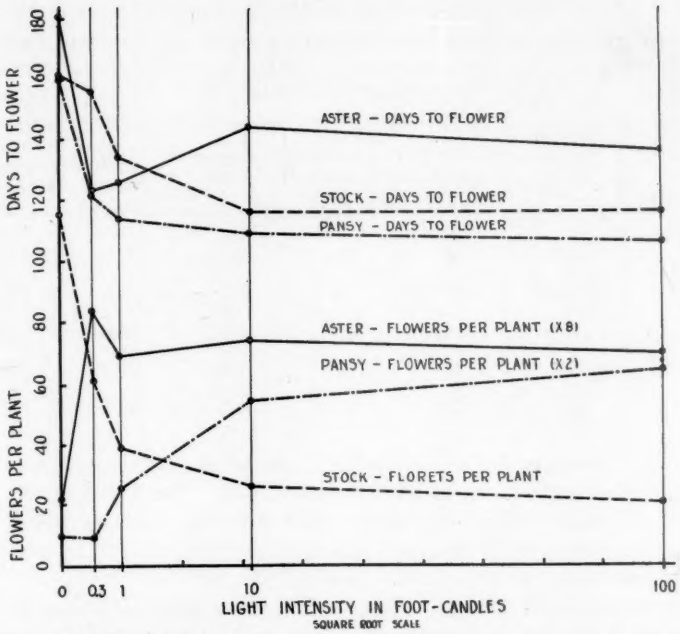
DAYS TO FLOWER
FLOWERS PER PLANT
ASTER - DAYS TO FLOWER
STOCK - DAYS TO FLOWER
PANSY - DAYS TO FLOWER
ASTER - FLOWERS PER PLANT (X8)
PANSY - FLOWERS PER PLANT (X2)
STOCK - FLORETS PER PLANT
0 0.3 1 10 100
LIGHT INTENSITY IN FOOT-CANDLES
SQUARE ROOT SCALE

Fig. 3. Effect of light intensity of supplemental irradiation on the flowering of pansy, stock, and aster.

## TABLE III

EFFECT OF INTENSITY OF LIGHT ON WEIGHT OF PANSY, VARIETY GIANT SWISS BLUE, SEEDED AUGUST 10, 1933. IRRADIATION BEGUN 8 WEEKS AFTER SEEDING FOR 49 DAYS FROM OCTOBER 10 TO NOVEMBER 28, 1933. AGE AT HARVEST, 110 DAYS

| LIGHT INTENSITY FOOT CANDLES | NUMBER PLANTS | TOPS | | ROOTS | TOTAL | | TOP/ROOT RATIO |
|---|---|---|---|---|---|---|---|
| | | FRESH WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT RATIO | |
| | | *gm.* | *gm.* | *gm.* | *gm.* | | |
| 100 | 14 | 8.0 | 0.76 | 0.15 | 0.91 | 1.25 | 5.2 |
| 10 | 11 | 7.9 | 0.80 | 0.23 | 1.03 | 1.42 | 3.5 |
| 1 | 17 | 6.2 | 0.81 | 0.28 | 1.09 | 1.50 | 2.9 |
| 0.3 | 15 | 5.3 | 0.63 | 0.26 | 0.89 | 1.22 | 2.4 |
| Control | 15 | 3.95 | 0.52 | 0.25 | 0.73 | 1.0 | 2.0 |

TABLE IV

EFFECT OF INTENSITY OF LIGHT ON FLOWERING OF STOCK, VARIETY LILAC LAVENDER, IMPROVED BISMARCK BRANCHING, SEEDED SEPTEMBER 14, 1933. IRRADIATION BEGUN 4 WEEKS AFTER SEEDING ON OCTOBER 10, 1933

| LIGHT INTENSITY FOOT CANDLES | NUMBER DAYS IRRADIATION | NUMBER PLANTS | AVERAGE NUMBER FLORETS PER PLANT | FLORET RATIO | AVERAGE STEM LENGTH | TOTAL TIME TO FLOWER |
|---|---|---|---|---|---|---|
| | | | | | *cm.* | *days* |
| 100 | 130 | 23 | 20.3 | 0.18 | 74.7 | 116 |
| 10 | 116 | 22 | 26.3 | 0.23 | 76.1 | 116 |
| 1 | 130 | 22 | 38.7 | 0.34 | 69.3 | 134 |
| 0.3 | 137 | 22 | 60.9 | 0.53 | 89.0 | 156 |
| Control | | 22 | 114.9 | 1.0 | 71.8 | 161 |

the lowest intensity of 0.3 foot candle produced marked reproductive and morphological responses in all three of these plants. The difference between 10 and 100 foot candles in inducing earlier flowering, affecting flower production and the height of the plant, was relatively slight in all cases, indicating the presence of limiting factors below 10 foot candles.

The number of flowers produced by pansy was hardly influenced by 0.3 foot candle, although this value did effect considerably earlier flowering. One foot candle brought about a much more definite response, with a flower production of two and one-half times that of the control and with still earlier

TABLE V

EFFECT OF INTENSITY OF LIGHT ON WEIGHT OF STOCK, VARIETY LILAC LAVENDER, IMPROVED BISMARCK BRANCHING, SEEDED SEPTEMBER 14, 1933. IRRADIATION BEGUN 4 WEEKS FROM SEEDING FOR 72 DAYS FROM OCTOBER 10 TO DECEMBER 21, 1933. AGE AT HARVEST, 98 DAYS

| LIGHT INTENSITY FOOT CANDLES | NUMBER PLANTS | TOPS | | ROOTS | TOTAL | | TOP/ ROOT RATIO | AVERAGE HEIGHT |
|---|---|---|---|---|---|---|---|---|
| | | FRESH WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT RATIO | | |
| | | *gm.* | *gm.* | *gm.* | *gm.* | | | *cm.* |
| 100 | 22 | 6.4 | 0.86 | 0.14 | 1.01 | 1.26 | 6.1 | 35.8 |
| 10 | 16 | 7.7 | 1.1 | 0.20 | 1.29 | 1.6 | 5.6 | 33.2 |
| 1 | 24 | 7.4 | 0.79 | 0.17 | 0.95 | 1.2 | 4.7 | 20.0 |
| 0.3 | 17 | 8.5 | 0.86 | 0.20 | 1.07 | 1.34 | 4.3 | 20.2 |
| Control | 25 | 5.75 | 0.64 | 0.16 | 0.80 | 1.0 | 4.0 | 10.2 |

## TABLE VI

EFFECT OF INTENSITY OF LIGHT ON FLOWERING OF ASTER, VARIETY HEART OF FRANCE, SEEDED SEPTEMBER 6, 1933. IRRADIATION BEGUN 4 WEEKS FROM SEEDING ON OCTOBER 10, 1933

| LIGHT INTENSITY FOOT CANDLES | NUMBER DAYS IRRADIATION | NUMBER PLANTS | AVERAGE NUMBER FLOWERS PER PLANT | FLOWER RATIO | AVERAGE FLOWER DIAMETER | ABNORMAL FLOWERS | AVERAGE STEM LENGTH | TOTAL TIME TO FLOWER |
|---|---|---|---|---|---|---|---|---|
| | | | | | *cm.* | *%* | *cm.* | *days* |
| 100 ..... | 153 | 20 | 8.8 | 3.3 | 8.1 | 0.0 | 21.2 | 136 |
| 10 ..... | 153 | 20 | 9.2 | 3.4 | 8.1 | 0.0 | 17.1 | 144 |
| 1 ..... | 148 | 20 | 8.7 | 3.2 | 7.4 | 0.0 | 15.7 | 126 |
| 0.3 ... | 141 | 20 | 10.6 | 3.9 | 4.4 | 0.0 | 12.7 | 122 |
| Control ... | ..... | 20 | 2.7 | 1.0 | 6.1 | 64.2 | 17.5 | 181 |

flowering. With 10 foot candles, twice the number of flowers occurred as in the 1 foot candle plot. This is a flower ratio of 5.5 as compared with the control. Increasing the intensity ten times raised the ratio only to 6.6. The artificial radiation resulted in slightly longer stems and smaller flowers. The relationships are shown in figure 3.

The variety of stock used was of the branching type which ordinarily

## TABLE VII

EFFECT OF INTENSITY OF LIGHT ON WEIGHT OF ASTER, VARIETY HEART OF FRANCE, SEEDED SEPTEMBER 6, 1933. IRRADIATION BEGUN 4 WEEKS FROM SEEDING FOR 78 DAYS FROM OCTOBER 10 TO DECEMBER 27, 1933. AGE AT HARVEST, 112 DAYS

| LIGHT INTENSITY FOOT CANDLES | NUMBER PLANTS | TOPS | | ROOTS | TOTAL | | TOP/ ROOT RATIO | AVERAGE HEIGHT |
|---|---|---|---|---|---|---|---|---|
| | | FRESH WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT RATIO | | |
| | | *gm.* | *gm.* | *gm.* | *gm.* | | | *cm.* |
| 100 ..... | 27 | 7.1 | 0.97 | 0.54 | 1.51 | 2.3 | 1.8 | 17.1 |
| 10 ..... | 20 | 8.3 | 1.06 | 0.41 | 1.47 | 2.3 | 2.6 | 17.0 |
| 1 ..... | 30 | 9.6 | 1.21 | 0.61 | 1.81 | 2.8 | 2.0 | 17.6 |
| 0.3 ... | 30 | 8.3 | 0.99 | 0.44 | 1.44 | 2.2 | 2.2 | 14.6 |
| Control ... | 20 | 3.3 | 0.41 | 0.23 | 0.65 | 1.0 | 1.8 | Rosette |

bears florets on each of its branches; therefore the number of florets is somewhat of an index to the branching condition. Treatment with 100 foot candles gave a columnar flowering stem with the florets somewhat more widely spaced than in the other conditions. There were small undeveloped branches present but these failed to produce florets or leaves. The branching habit was more definitely manifest as the light intensity was decreased. The most profusely branched plants occurred under the short day or control condition, with a marked decrease in branching brought about by the addition of only 0.3 foot candle supplemental light. Those plants under long days bloomed more quickly than did those under short days, the higher intensities producing the earliest flowers. Figure 4 shows the effect of intensity of light on stock as to height and earliness of flowering.

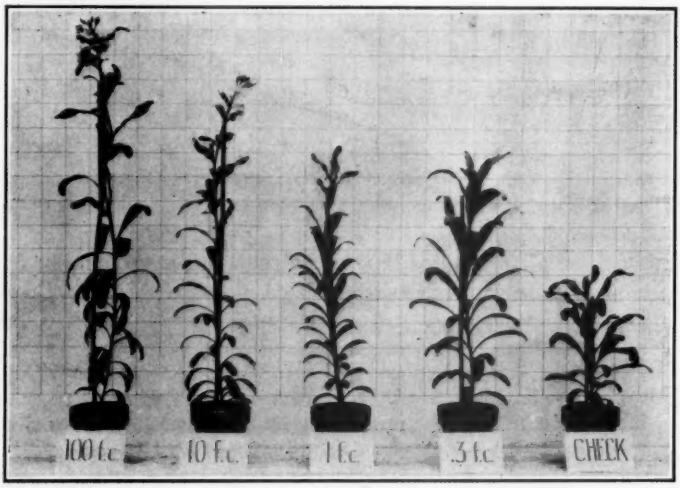


FIG. 4. Effect of light intensity of supplemental irradiation on stock.

Aster showed the most marked response to 0.3 foot candle of the three species used (fig. 5). This group flowered first and produced the largest number of blooms per plant. The higher intensities brought about slightly later flowering and lower production but considerably larger blossoms. Approximately two months after the plants under the lowest intensity bloomed, the control plants flowered. Sixty-four per cent. of the flowers in this group were abnormal, with the petals twisted and curled into small compact heads. The flower diameter given for the controls is based on the measurement of normal flowers only. The stem length of the flowers varied with intensity, the highest intensity producing the longest stems.

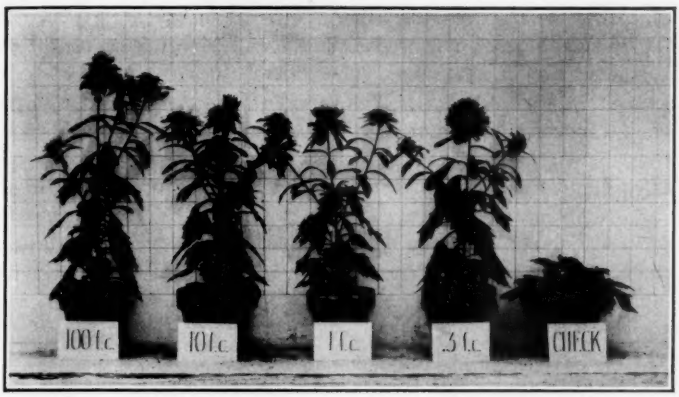


FIG. 5. Effect of light intensity of supplemental irradiation on aster.

When it was noted that an intensity of 0.3 foot candle was having such a marked effect on aster seedlings, it was decided to use a still lower intensity. Three plots of aster were benched, including the following treatments: control, 10 foot candles, and 0.1 foot candle. The plants were seeded at a later

FIG. 6. Effect of very low light intensity of supplemental irradiation on aster, seeded September 23, 1933. Irradiation from November 4, 1933. Photographed March 26, 1934. Age to date, 111 days.

date than those in the previous experiment. It can be seen from figure 6 that 0.1 foot candle has a very definite effect in increasing height and in bringing about earlier flowering. The control showed no sign of blooming at the time of the photograph. The 0.1 foot candle produced a large percentage of abnormal flowers of the same type as did the controls at a later date.

Tables III, V, and VII present the height and weight data of pansy, stock, and aster obtained by harvesting a portion of the plants of each plot before any of them reached flowering. The height data show much the same relationships as presented in figures 4 and 5 for aster and stock. Stock, being a less sensitive plant than aster to daylength, showed less difference in height between the control and 0.3 foot candle groups and a definite increase in height with an increase in light intensity. The height of aster under 0.3 foot candle was almost as great as that under 100 foot candles, even though the light intensity was 300 times as great in the latter case. The control

TABLE VIII

EFFECT OF WAVELENGTH OF LIGHT ON FLOWERING OF PANSY, VARIETY GIANT SWISS BLUE, SEEDED AUGUST 10, 1933. IRRADIATION BEGUN 8 WEEKS AFTER SEEDING FOR 149 DAYS FROM OCTOBER 10, 1933, TO MARCH 8, 1934

| COLOR OF LIGHT | WAVELENGTH | NUMBER PLANTS | AVERAGE NUMBER FLOWERS PER PLANT | FLOWER RATIO | AVERAGE FLOWER DIAMETER | AVERAGE STEM LENGTH | TOTAL TIME TO FLOWER |
|---|---|---|---|---|---|---|---|
| | *mμ* | | | | *cm.* | *cm.* | *days* |
| Blue | 380–510 | 14 | 0.8 | 0.42 | 5.6 | 11.0 | 198 |
| Green | 455–550 | 14 | 1.2 | 0.63 | 5.8 | 11.1 | 202 |
| Yellow | 530–650 | 14 | 1.7 | 0.90 | 6.1 | 10.9 | 198 |
| Orange-red | 650– – | 14 | 19.1 | 10.0 | 5.1 | 10.2 | 114 |
| Red | 680– – | 14 | 13.2 | 6.95 | 7.5 | 12.2 | 114 |
| Extreme red | 720– – | 14 | 3.9 | 2.02 | 5.4 | 13.0 | 155 |
| Infra-red* | 800– – | 14 | — | 0.74 | — | — | 117 |
| Control | – | 14 | 1.9 | 1.0 | 5.6 | 8.5 | 191 |
| Infra-red* | 800– – | 14 | 3.1 | 0.74 | 5.3 | 9.7 | 117 |
| Control* | – | 14 | 4.2 | 1.0 | 6.0 | 10.0 | 191 |

* Plants in the infra-red plot were grown under conditions slightly different from those of the other filter plots. The data for infra-red irradiated plants are presented with those of its separate control plot and are comparable with the others in ratio values only.

aster plants, at the time of this harvest, were still in the rosette stage. The stem length measurements presented in the tables of flowering data for stock and aster do not show these same relationships because the data of these tables were obtained at the time the plants came into flower, regardless of the date. Thus in the case of stock, the control plants at any given time before the 100 foot candle plot came into bloom were much shorter. By the time the controls flowered, however, they had attained a height comparable with that of the lighted groups. No height data were recorded for pansy because of the prostrate growth habit of the plant, but it was noted that in all the lighted plots the internodes were longer than in the controls.

In all cases, regardless of the species, additional supplemental light resulted in a greater total dry weight per plant than the weight for the plants subjected to the natural short days. In general, the differences in dry weight between the various lighted plots appear to show no significant trends. The increases in dry weight of the lighted plants over the controls

## TABLE IX

EFFECT OF WAVELENGTH OF LIGHT ON WEIGHT OF PANSY, VARIETY GIANT SWISS BLUE, SEEDED AUGUST 10, 1933. IRRADIATION BEGUN 8 WEEKS AFTER SEEDING FOR 49 DAYS FROM OCTOBER 10 TO NOVEMBER 28, 1933. AGE AT HARVEST, 110 DAYS

| COLOR OF LIGHT | WAVE-LENGTH | NUM-BER PLANTS | TOPS | | ROOTS | TOTAL | | TOP/ ROOT RATIO |
|---|---|---|---|---|---|---|---|---|
| | | | FRESH WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT RATIO | |
| | mμ | | gm. | gm. | gm. | gm. | | |
| Blue | 380–510 | 17 | 4.3 | 0.57 | 0.24 | 0.81 | 0.9 | 2.4 |
| Green | 455–550 | 19 | 4.4 | 0.42 | 0.23 | 0.66 | 0.7 | 1.8 |
| Yellow | 530–650 | 14 | 3.8 | 0.55 | 0.24 | 0.79 | 0.9 | 2.3 |
| Orange-red | 650– – | 15 | 6.1 | 0.64 | 0.26 | 0.91 | 1.0 | 2.4 |
| Red | 680– – | 17 | 5.0 | 0.58 | 0.25 | 0.83 | 0.9 | 2.3 |
| Extreme red | 720– – | 14 | 4.1 | 0.49 | 0.24 | 0.73 | 0.8 | 2.0 |
| Infra-red* | 800– – | 14 | — | — | — | — | 1.3 | — |
| Control | – | 17 | 4.8 | 0.52 | 0.39 | 0.91 | 1.0 | 1.3 |
| Infra-red* | 800– – | 14 | 4.6 | 0.70 | 0.26 | 0.96 | 1.3 | 2.7 |
| Control* | – | 16 | 3.9 | 0.49 | 0.26 | 0.75 | 1.0 | 1.9 |

* See footnote to table VIII.

were due largely to increases in the weight of the tops. Pansy and stock showed a general increase in top-root ratio with an increase of light intensity.

### Wavelength series

The orange and red portions of the visible spectrum produced the most conspicuous influence on the flowering responses of the three species studied, as compared with results secured with the blue, green, yellow, and infra-red portions of the spectrum employed. Prolonging the day with orange-red radiation resulted in the greatest number of flowers with pansy and aster and the minimum of lateral branches, and therefore of florets, with stock. These relationships are brought out in tables VIII, X, and XII, and in

TABLE X

Effect of wavelength of light on flowering of stock, variety Lilac Lavender, Improved Bismarck Branching, seeded September 10, 1933. Irradiation begun 4 weeks after seeding from October 10, 1933

| Color of light | Wavelength | Number days of irradiation | Number plants | Average number florets per plant | Floret ratio | Average stem length | Total time to flower |
|---|---|---|---|---|---|---|---|
| | *mμ* | | | | | *cm.* | *days* |
| Blue | 380–510 | 153 | 18 | 117.0 | 0.93 | 84.1 | 141 |
| Green | 455–550 | 153 | 22 | 103.9 | 0.82 | 75.5 | 130 |
| Yellow | 530–650 | 153 | 22 | 63.7 | 0.50 | 79.3 | 137 |
| Orange-red | 650– – | 130 | 23 | 27.7 | 0.22 | 87.6 | 102 |
| Red | 680– – | 137 | 23 | 39.0 | 0.31 | 74.1 | 98 |
| Extreme red | 720– – | 153 | 22 | 55.5 | 0.44 | 80.3 | 118 |
| Infra-red* | 800– – | 153 | 21 | — | 0.69 | — | 130 |
| Control | – | — | 15 | 126.6 | 1.0 | 68.3 | 130 |
| Infra-red* | 800– – | 153 | 21 | 87.2 | 0.69 | 65.3 | 130 |
| Control* | – | — | 23 | 120.3 | 1.0 | 72.4 | 141 |

* See footnote to table VIII.

figure 7. The earliest flowering for pansy and stock occurred in plants radiated with these wavelengths. In the case of aster, which is by far the most sensitive of the three plants to daylength, all regions from red to blue induced flowering at nearly the same time. It is interesting to note that the greatest proportion of abnormal flowers of aster occurred under the control, blue, extreme red, and infra-red bands, with the smallest number present in

## TABLE XI

EFFECT OF WAVELENGTH OF LIGHT ON WEIGHT OF STOCK, VARIETY LILAC LAVENDER, IMPROVED BISMARCK BRANCHING, SEEDED SEPTEMBER 10, 1933. IRRADIATION BEGUN 4 WEEKS AFTER SEEDING FOR 72 DAYS FROM OCTOBER 10 TO DECEMBER 21, 1933. AGE AT HARVEST, 102 DAYS

| COLOR OF LIGHT | WAVE-LENGTH | NUMBER PLANTS | TOPS | | ROOTS | TOTAL | | TOP/ ROOT RATIO | AVER-AGE HEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | | FRESH WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT RATIO | | |
| | *mμ* | | *gm.* | *gm.* | *gm.* | *gm.* | | | *cm.* |
| Blue | 380–510 | 23 | 4.4 | 0.48 | 0.11 | 0.58 | 0.7 | 4.4 | 11.1 |
| Green | 455–550 | 19 | 5.7 | 0.57 | 0.12 | 0.70 | 0.9 | 4.6 | 12.4 |
| Yellow | 530–650 | 30 | 6.0 | 0.58 | 0.12 | 0.70 | 0.9 | 4.7 | 12.1 |
| Orange-red | 650– – | 26 | 7.0 | 0.73 | 0.13 | 0.86 | 1.1 | 5.8 | 29.6 |
| Red | 680– – | 24 | 6.6 | 0.70 | 0.14 | 0.83 | 1.05 | 5.2 | 25.6 |
| Extreme red | 720– – | 27 | 7.1 | 0.75 | 0.14 | 0.88 | 1.1 | 5.5 | 22.0 |
| Infra-red* | 800– – | 33 | — | — | — | — | — | — | — |
| Control | – | 23 | 5.7 | 0.63 | 0.16 | 0.80 | 1.0 | 3.9 | 10.2 |
| Infra-red* | 800– – | 33 | 5.1 | 0.59 | 0.16 | 0.75 | 0.9 | 3.7 | 8.8 |
| Control* | – | 24 | 5.9 | 0.61 | 0.19 | 0.80 | 1.0 | 3.3 | 10.1 |

* See footnote to table VIII.

## TABLE XII

EFFECT OF WAVELENGTH OF LIGHT ON FLOWERING OF ASTER, VARIETY HEART OF FRANCE, SEEDED SEPTEMBER 6, 1933. IRRADIATION BEGUN 4 WEEKS AFTER SEEDING FROM OCTOBER 10, 1933

| COLOR OF LIGHT | WAVE-LENGTH | NUMBER DAYS OF IRRADI-ATION | NUMBER PLANTS | AVERAGE NUMBER FLOWERS PER PLANT | FLOWER RATIO | AVERAGE FLOWER DIAMETER | ABNORMAL FLOWERS | AVERAGE STEM LENGTH | TOTAL TIME TO FLOWER |
|---|---|---|---|---|---|---|---|---|---|
| | *mμ* | | | | | *cm.* | *%* | *cm.* | *days* |
| Blue | 380–510 | 153 | 19 | 4.0 | 1.4 | 5.2 | 44.5 | 8.4 | 158 |
| Green | 455–550 | 153 | 20 | 6.3 | 2.1 | 6.4 | 10.1 | 13.6 | 145 |
| Yellow | 530–650 | 153 | 20 | 8.4 | 2.8 | 7.0 | 0.0 | 15.2 | 149 |
| Orange-red | 650– | 153 | 20 | 9.5 | 3.2 | 7.8 | 1.6 | 15.9 | 155 |
| Red | 680– | 141 | 20 | 5.1 | 1.7 | 7.2 | 3.8 | 13.4 | 151 |
| Extreme red | 720– | 153 | 20 | 4.8 | 1.6 | 6.1 | 35.1 | 13.3 | 174 |
| Infra-red* | 800– | 153 | 19 | ..... | 2.0 | ..... | ..... | ..... | 181 |
| Control | – | ..... | 20 | 2.95 | 1.0 | 6.5 | 62.7 | 17.6 | 193 |
| Infra-red* | 800– | 153 | 19 | 4.8 | 2.0 | 6.0 | 48.0 | 15.4 | 181 |
| Control* | – | ..... | 20 | 2.4 | 1.0 | 5.7 | 65.7 | 17.4 | 209 |

* See footnote to table VIII.

## TABLE XIII

EFFECT OF WAVELENGTH OF LIGHT ON WEIGHT OF ASTER, VARIETY HEART OF FRANCE, SEEDED SEPTEMBER 6, 1933. IRRADIATION BEGUN 4 WEEKS AFTER SEEDING FOR 78 DAYS FROM OCTOBER 10 TO DECEMBER 27, 1933. AGE AT HARVEST, 112 DAYS

| COLOR OF LIGHT | WAVE-LENGTH | NUMBER PLANTS | TOPS | | ROOTS | TOTAL | | TOP/ROOT RATIO | AVERAGE HEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | | FRESH WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT | DRY WEIGHT RATIO | | |
| | *mμ* | | *gm.* | *gm.* | *gm.* | *gm.* | | | *cm.* |
| Blue | 380–510 | 29 | 6.3 | 0.66 | 0.25 | 0.92 | 1.4 | 2.6 | Rosette |
| Green | 455–550 | 22 | 7.9 | 0.92 | 0.31 | 1.23 | 1.9 | 3.0 | 8.1 |
| Yellow | 530–650 | 30 | 8.1 | 0.95 | 0.35 | 1.30 | 2.0 | 2.7 | 10.7 |
| Orange-red | 650–..... | 25 | 7.8 | 0.96 | 0.31 | 1.27 | 1.9 | 3.1 | 12.5 |
| Red | 680–..... | 24 | 7.7 | 0.99 | 0.44 | 1.43 | 2.2 | 2.3 | 13.5 |
| Extreme red | 720–..... | 26 | 6.5 | 0.77 | 0.33 | 1.10 | 1.7 | 2.3 | 4.0 |
| Infra-red* | 800–..... | 30 | ..... | ..... | ..... | ..... | 1.2 | ..... | ..... |
| Control | – | 16 | 3.7 | 0.42 | 0.24 | 0.66 | 1.0 | 1.7 | Rosette |
| Infra-red* | 800–..... | 30 | 3.9 | 0.52 | 0.26 | 0.76 | 1.2 | 2.0 | Rosette |
| Control* | – | 25 | 2.9 | 0.41 | 0.23 | 0.64 | 1.0 | 1.8 | Rosette |

* See footnote to table VIII.

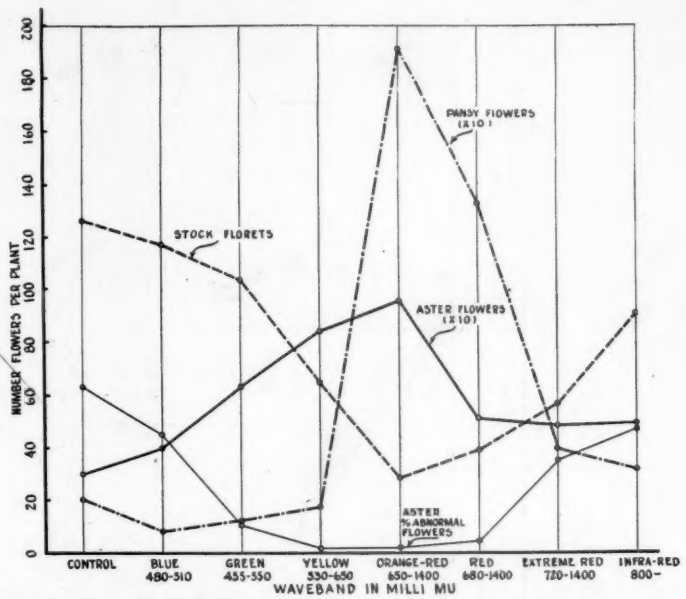


FIG. 7. Effect of wavelength of supplemental irradiation on the flowering of pansy, stock, and aster.

the yellow, orange-red, and red radiated groups. During the course of the experiment, it was observed that the relatively high blue energy tended to produce an apparent stunting effect on the plants. This was especially true with aster, in which the frequency of abnormal flowers was high and the flower production very low.

Owing to lack of space on the main bench, the infra-red plot was placed in another portion of the same house, adjacent to its separate control plot. The growing conditions were slightly different from those of the other filter plots. The data indicate that slight responses were obtained with regard to the flowering of the plants radiated with infra-red. In the early stages of growth of aster, however, none of the typical photoperiodic effects were noted, especially with regard to the color of leaves, development of internodes, and the general tendency of the leaves to tilt upward, which characteristics have been generally observed whenever this plant received radiation which resulted in definitely earlier flowering. It appears likely that the responses were due more to the light which trickled through the cracks of the glass of the filters during the early period, before filters were developed which would withstand the heat strain occurring during the long irradiation periods. The infra-red filter also passed very slight traces of visible red

radiation. Further, a definite heating effect of the leaf tissues was noted under this radiation condition. It is difficult to interpret the results obtained as to whether they were due to infra-red, to trickled visible radiations,

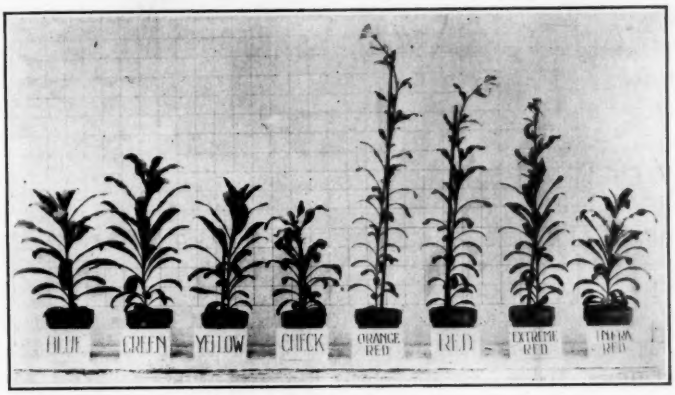


FIG. 8. Effect of wavelength of supplemental irradiation on stock.

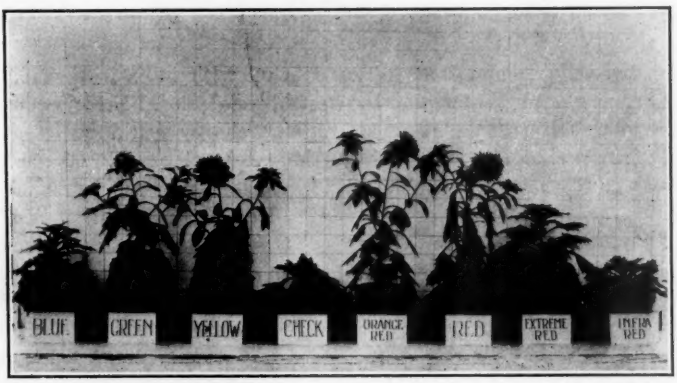


FIG. 9. Effect of wavelength of supplemental irradiation on aster.

or to heating. Figures 8 and 9 show the gross morphological responses of stock and aster to filtered radiation.

The height and weight data (tables IX, XI, and XIII) amplify the relationships shown by the flowering responses. The tallest plants in all cases occurred in the orange-red and red plots, while the shortest ones were in the control and infra-red, those in the blue being only slightly larger. These

relationships for stock and aster are clearly shown in figures 8 and 9, although the photographs were taken at a considerably later time than the harvest date of the plants for height and weight data. In general, the weight of the tops was also greatest in the orange and red regions. The total dry weights of radiated plants, however, were only very slightly greater than those of the controls and the differences may not be very significant for pansy and stock.

## Discussion

The results indicate that large quantities of radiant energy are not necessary for the operation of the photoperiodic mechanism and that it is a process which is limited in its functioning by other factors which are probably internal. The three species varied markedly in the minimum value of intensity which could produce a normal transformation of the plant from the vegetative to the reproductive state. In the case of aster, this value is in the neighborhood of 0.3 foot candle of incandescent lamp radiation. For stock and pansy, the limit appears to be in the neighborhood of 10 foot candles. Intensities beyond these threshold values produce relatively little further increase in reproductive response. The remarkable sensitivity of China aster to low radiation intensities is shown by the induction of early flowering by intensities as low as 0.1 foot candle, which is only twice the intensity of bright moonlight as observed in the vicinity of Lafayette, Indiana.

The dry weights of all the plants treated with intensities of 0.3 foot candle or more were greater than the control weights. It hardly seems conceivable that this small amount of radiant energy could be capable of directly influencing photosynthesis to the extent observed. Even during dark winter weather, the plants received, on the average, at least 5000 foot candle hours of solar radiation per day. During the night, the 0.3 foot candle plot received not more than about 3 foot candle hours of incandescent lamp radiation. The resultant greater dry weight may be due to an indirect effect of the nightly radiation period on the photosynthetic activity during the following day, possibly through accelerated removal during the night of the products of photosynthesis accumulated during the day. Such a mechanism has been postulated by TINCKER and others on the basis of other observations.

The higher top-root ratios of the radiated plants indicate that the additional products of photosynthesis are largely utilized in the tops without translocation to the root system. RAMALEY (**10**) observed a smaller phloem in plants subjected to long days. GARNER and ALLARD (**1**), TINCKER (**14**), and others observed reduced tuberization under long days and a general tendency toward less storage of food materials in the underground portions of the plant.

In the present investigations, as in the earlier one, the orange and red regions of the visible spectrum produced the most marked photoperiodic responses. The results intimate that the photoperiodic mechanism has a maximum spectral sensitivity in the vicinity of 650 to 720 mμ. The green and yellow regions from 450 to 650 mμ show little effect in the case of pansy and stock. Aster, which is exceptionally sensitive to low intensity radiation as applied to prolong the day, responded almost as well to the yellow and green regions as to the other bands. Such a species as China aster is sensitive enough to the yellow and green to respond with earlier flowering, with the high intensities of radiation used, as compared with the controls. From the relatively slight response generally obtained with the extreme red of wavelengths longer than 720 mμ, the sensitivity curve seems to drop off more quickly toward the longer wavelengths than toward the shorter. The blue band of radiation of the intensity employed produced little effect in any case and there was some evidence of stunting and delay in flowering with pansy and stock.

## Summary

1. Experiments on the effect of intensity of incandescent lamp radiation and wavelengths of artificial radiation used to prolong the day over pansy (*Viola tricolor*), stock (*Matthiola incana*), and China aster (*Callistephus chinensis*) are reported. The intensities used were 100, 10, 1, 0.3, and 0.1 foot candles. The wavelength bands included blue (380–510 mμ), green (455–550 mμ), yellow (530–650 mμ), orange-red (650– – – mμ), red (680– – – mμ), extreme red (720– – – mμ), and infra-red (800– – – mμ).*

2. In the intensity investigations, little difference in response was secured between 100 and 10 foot candles and a very definite photoperiodic effect was produced by 0.3 foot candle and as low as 0.1 foot candle with China aster. An increase over the control in dry weight was produced in all three species under all of the intensities of artificial radiation employed. An increase in top-root ratio was also noted in pansy and stock with an increase in light intensity.

3. Orange and red radiation caused the most marked photoperiodic response in pansy, stock, and aster. The earliest blooming with pansy and stock and the largest number of flowers with all three were occasioned by these bands, especially the orange-red. The greatest departure from the branching habit of the variety of stock used was noted under these wavelengths. Little effect on flowering time was observed in the case of pansy and stock with blue, green, and yellow radiation. With aster, all wavelengths of additional light brought about earlier flowering than in the control plants. In all three species, the top-root ratio was greater with all wavelengths than in the control, the greatest ratio being under the orange-red. Greater fresh weight also occurred under orange and red radiation.

* Upper limits uncertain.

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# NITROGEN NUTRITION OF SUGAR CANE

U. K. Das

(WITH TWENTY-THREE FIGURES)

## Introduction

In the intensive culture of sugar cane in Hawaii nitrogen plays a key rôle. It is largely through liberal increases in nitrogenous fertilization that the cane industry has been able to more than double its annual output in the last twenty years. On the irrigated plantations of Hawaii, an application of two to three hundred pounds of nitrogen per acre per crop is the usual practice. In terms of nitrogen-containing fertilizer like ammonium sulphate, this amount would represent an application of over half a ton of fertilizer per acre.[1] So enormous is the capacity of the cane plant to take up this nitrogen that little is left in the soil and each succeeding crop has to be fertilized equally heavily to maintain the yield, which runs about eighty to a hundred tons of millable cane per acre.

These enormous applications of nitrogen have not, however, been without drawbacks; while the cane yield has increased more than 100 per cent., its quality, as measured by the number of tons of cane required to make a ton of sugar, has actually decreased about 15 per cent. It is estimated that had there been no loss in quality, the sugar industry might have netted several million dollars more every year. The question is therefore being seriously asked, Is high nitrogen fertilization incompatible with good quality? If so, is there any way of preventing at least the extent to which quality will deteriorate? Indeed, no further increases in the nitrogenous fertilization can be contemplated without an answer to these questions.

The present study was undertaken for the purpose of gathering fundamental information on the effects of widely varied amounts of nitrogen on the yield of cane and on the physico-chemical characteristics of the juice. Without such information, all efforts toward improvement of quality may not gain their objective.

There is a considerable body of literature on the nitrogen nutrition of different plants, but so far as the writer is aware no such study appears to have been undertaken previously with sugar cane. In Hawaii and in other cane-producing countries, innumerable field experiments have been run in order to determine the most profitable amount of nitrogen to apply. The data on final yields thus gathered, while of undoubted agricultural

[1] In marked contrast to other tropical cane regions, Hawaii's mild and almost subtropical climate makes it necessary to grow sugar cane as a biennial crop. The high applications of nitrogen are therefore intended to maintain the crop over a period of two years.

value, do not give any insight into the influence of nitrogen on the metabolic processes of the plant.

### Plan and experimental procedure

It was proposed to study three widely different amounts of nitrogen, one considered to be very low, one known from field experiments to be about optimum, and one very high. It was felt that only through such exaggeration of differences would the metabolic effects of nitrogen on cane plants be clearly brought out.

A uniform area of land at the Experiment Station in Honolulu was chosen as the locale of the study. The land was thoroughly prepared and divided into three sections, each being separated from the other by 10 feet. Each section was further divided into nineteen rows, each row 25 feet long and 5 feet wide.

PLANTING MATERIAL, TAGGING OF PLANTS.—On June 21, 1933, uniform seed pieces[2] of the sugar cane variety H 109 were planted. Germination of new shoots started within three weeks. At the end of five weeks, when germination was considered to be complete, the weak shoots in each row were weeded out, leaving only the more uniform and vigorous ones. In order to eliminate variations arising from differences in the original stand, the same number of shoots, namely sixty-five, were kept in each row. These original shoots arising from the seed piece are in this paper called mother stalks or stalks of the first order. Each of these stalks was numbered in sequence with a weatherproof tag so that at any later period its identity would be precisely known. The tillers that came up between the date of marking the mother stalks and the third month are called daughter stalks or stalks of the second order. All the second order stalks were similarly tagged. These precautions were undertaken to facilitate sampling procedures when the heavy growth of cane would render separation of the various orders impossible by the eye alone.

FERTILIZATION.—The total amounts of fertilizer applied were as follows:

Low nitrogen plot: 133.30 pounds of N from ammonium sulphate,
200 pounds of $P_2O_5$ from superphosphate,
200 pounds of $K_2O$ from potassium sulphate.
Medium nitrogen plot: 266.65 pounds of N; $P_2O_5$, and $K_2O$ same as in the low N plot.
High nitrogen plot: 645 pounds of N; $P_2O_5$, and $K_2O$ same as in the other treatments.

The phosphate and potash fertilizers were applied in one dose at the time of planting but the nitrogen was applied in seven doses:

[2] Sugar cane is propagated vegetatively by planting a section of the stalk, preferably a young stalk. Such sections are referred to as seed pieces, as distinguished from true seeds.

SCHEME OF NITROGEN FERTILIZATION

| AGE OF CROP | LOW N | MEDIUM N | HIGH N |
|---|---|---|---|
| 0 month (with seed) | 25 pounds | 50 pounds | 75 pounds |
| 3½ " | 25 " | 50 " | 75 " |
| 6 " | 16.66 " | 33.33 " | 99 " |
| 8½ " | 16.66 " | 33.33 " | 99 " |
| 10½ " | 16.66 " | 33.33 " | 99 " |
| 12½ " | 16.66 " | 33.33 " | 99 " |
| 14½ " | 16.66 " | 33.33 " | 99 " |
| Total | 133.30 " | 266.65 " | 645 " |

When the cane was young and the rows physically accessible, the nitrogen was applied by hand. After nine months the cane field was such a jungle of growth that application by hand was impossible; the last three applications were therefore applied in the irrigation water.

IRRIGATION.—Water was applied at the rate of 2 inches per acre per irrigation, two applications per week, irrespective of season.[3] Sixteen to 18 inches of water thus applied every month is considered more than sufficient for the requirements of the plant. Water is thereby removed as a limiting factor or as a source of variation.

HARVESTING PROCEDURE.—Beginning at the age of three and one-half months and every second month thereafter, one line of cane from each section was harvested. A day or two before the harvest, samples were taken from each line for physico-chemical determinations. These samples consisted of five first-order stalks taken 5 feet apart in the row and two or three second-order stalks growing in the immediate vicinity of the selected first-order stalks.

The first, second, and higher orders of stalks were segregated at each harvest. The dead and the dying leaves were removed from the stalks, which were then divided into two sections, the millable cane and the nonmillable top. The latter is that part of the cane which, in commercial practice, is left in the field. It consists of green blades, sheath, and very immature joints of cane. The millable cane was further divided into two sections: (1) the "green-leaf" cane, *i.e.*, that part of the millable stalk where some green or partly green leaves are still tightly adhering; (2) the "dry-leaf" or the "dead-leaf" section, where the joints are fully exposed and no leaves are adhering.

This manner of dividing the cane into three sections for the purpose of studying quality was first employed in our previous study (**10**) and repre-

[3] In the rainy season (precipitation about 3 inches per month) allowance was made for heavy showers so that the ground might not become too wet.

sents a departure from the usual practice in chemical studies of the cane plant. It will be obvious that the separation of the tissue in the various plant parts, although entailing much more work, must provide better information on the biochemical effects of nitrogen fertilization.

At this point it should be emphasized that although the separation of the dry-leaf section from the rest of the cane is more or less precise, the separation of the green-leaf cane from the non-millable top is much more subject to errors of judgment. What one person employed for cutting the cane might consider a non-millable joint, another might consider a part of the millable green-leaf cane. This empirical method of separation introduces a source of uncontrolled variation in the green-leaf and the top sections which is not present in the dry-leaf section.

## Agronomy

### General observations

Tillering was more pronounced in the high N plot than in the others, resulting in more stalks per line at harvest. At three months of age the low N plants looked the greenest and the cane in parts of the other two plots somewhat yellow. This was contrary to expectations. It so happens that the part of the area showing this yellowing had been cleared of growing cane only a short while before, while the areas in the low N plot and in parts of the other two treatments were fallow for several months. It is hard to say whether fallowing had any beneficial effect on the general metabolism and on nitrogen availability or its uptake by the plants. Analysis previous to planting showed about the same amount of total nitrogen in all the plots. Yet, and in spite of the lower application, the low N plants at three and one-half months were found to have actually a greater amount of nitrogen per row than those having the other two treatments. The percentage of nitrogen on the dry basis was, however, lower in the low N than in the medium or the high N plants. The greenness of the leaves at three and one-half months of age could not therefore be correlated with the percentage of nitrogen contained in the plants. Soon after the second application of nitrogen, all the plots looked equally green. At six months the high N plot had a deep green color; the medium and the low nitrogen plots were slightly less green. From the ninth month on, the low N plants looked yellowish green while the high N plants were deep bluish green.

The next marked difference between the treatments was observed in the matter of stand. The plants in the high N plot began to lodge at six months, those in the medium N plot at eight to nine months, and those in the low N plot were still erect at twelve to thirteen months. Thus the high N plot early became a mass of tangled growth with no possibility of

light penetrating to the soil, while the low N plot remained open and accessible to some light for a considerably longer period. Lodging is no doubt due to the increased succulence of the high N plants (see data on water relationships). WELTON (**34**) and BRADY (**4**) find lodging is associated in cereal with less lignified and sclerenchymatic tissue, which would imply that in the high N plants the woody supporting tissue is less developed than in the low N plants. It may also be, as RIPPEL and LUDWIG (**27**) have shown, that with a smaller rate of nitrogen application the ratio of the dry weight of the aerial part to the dry weight of the roots is less. Such relatively greater root production may also be a factor in the greater stability of the plants in the low N treatment.

### CHARACTER AND RATE OF GROWTH

The influence of increasing applications of nitrogen on the character of growth was studied on twenty selected mother stalks from each treatment. As the stalks were located in a single furrow well inside the field, they were subject to the same conditions of plant competition as the rest of the cane harvested from time to time in this experiment. This row of cane was treated in every other way exactly as the other rows. Beginning at the age of three and one-half months and every two weeks thereafter, the twenty selected stalks were measured in length and the rate of elongation obtained. On each occasion the number of new leaves formed since the last measurement was also recorded, as well as the length and width of the topmost fully developed leaf on each stalk of cane. The leaf measurements became rather infrequent after the seventh month because of physical difficulties in reaching them without disturbing the other cane.

An estimate was also made of the longevity of leaves after they reach the fully developed stage by noting the time it takes for them to pass from the green to the yellow stage and finally to decay and fall.

LENGTH, WIDTH, AND LONGEVITY OF LEAVES.—The length of the blade was measured from its point of junction with the sheath to the very tip. In sugar cane this tip tapers to a very fine point and is often broken off by the wind, so the end point has to be estimated. This involves a source of error which should be kept in mind in discussing the data. The width measurements were taken uniformly at a distance of 2 feet from the junction of the blade and the sheath and are much more trustworthy. In general, the maximum width of the leaf is to be found at about this distance from the sheath, but individual exceptions have sometimes been noted. The width and length may then be considered only in a semi-quantitative manner.

Figure 1 shows that the blade length increased in all the treatments up to January, 1934, age six months, following which there was a decline to a

more or less stable value which was maintained from the end of February to the end of September. There appeared to be a further decrease in length in the spring of 1935. The high N series appears to have the shortest blade while the medium N has the longest in the earlier months, although the differences largely disappear later. In view of the limitations of the data, it cannot be said with certainty whether the early differences are significant.

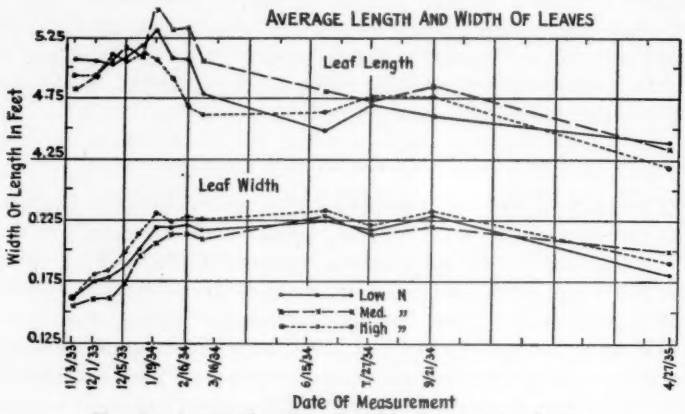



FIG. 1. Average length and width of sugar cane leaves.

In the case of leaf width, exactly the same trend is observed. The width also reaches a maximum at six months of age and then declines to a stable value between the eighth and the fourteenth month, the width showing a further decrease in the spring of 1935. The course of changes in leaf size is no doubt associated with the stage of development and the intensity of growth of the stalk.

It is generally known that the cane plant reaches the adult stage (millable cane) at about five to six months of age. VERRET and DAS (**33**) have shown that the rate of vegetative growth (elongation) in this variety of cane also reaches a maximum at the age of about six months.

That the leaf size may be a function both of the age of the plant and of nutritional conditions is indicated by the fact that as long as nitrogen application is continued the size of the leaf remains constant, and with its cessation the size again diminishes. The constancy of leaf dimensions between the eighth and the fourteenth month supports the similar observations of investigators in Java.

It will be noted with great interest that the treatment differences on width are the reverse of those on length. Here the high N series have the widest leaves and from the nature of the data the difference may be considered significant.

Data on longevity show that in general a leaf stays fully green about two months and the process of yellowing lasts about two weeks, so that the leaf is fully decayed in about two and one-half months. In other words, from the time a joint begins to develop to the time it forms a part of the dry leaf cane is only a matter of weeks.

RATE OF ELONGATION.—The underlying theories as well as the actual procedure of growth measurement in cane have been elaborated in previous publications of STENDER (**30**), VERRET and DAS (**33**), and others. Suffice it to say here that the length of the stalk is measured each time to the last visible "joint triangle," *i.e.*, the clearly defined junction of the blade and the sheath. The differences between the measurements show the exact elongation in that part of the cane which is not visible to the eye. The rate of elongation, therefore, reflects entirely the influences of current conditions.

Figure 2 (data in table I) shows the elongation in feet during two-week periods ending at the date noted. It should be emphasized again that the graph shows the rate and not the total growth as influenced by the seasonal or cultural conditions. From a fairly high point in October, 1933, the rate declines, with fluctuations, to a minimum value at the end of April, following which there is a tremendous increase, reaching a maximum in the middle of July. This high rate of growth is maintained until September, 1934, and is followed by a rapid decline. In the early months of 1935, the rate of length growth is even less than it was at the minimum point the previous April.

All three series grow at about an equal rate until the ninth month, *i.e.*, March, 1933, and then the low N series begins to lag behind the other two

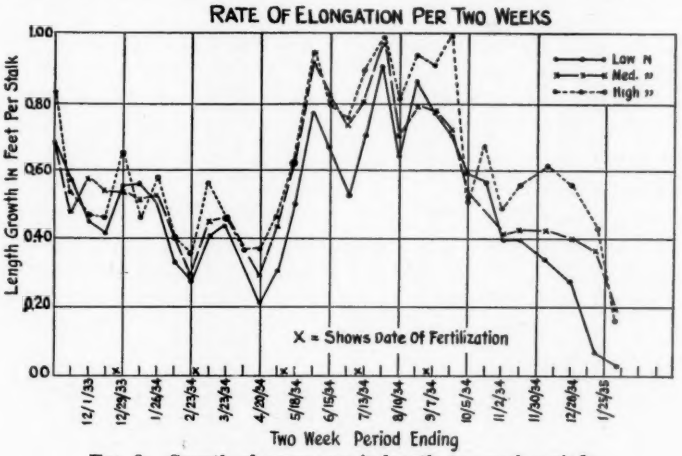



FIG. 2. Growth of sugar cane in length, two-week periods.

## TABLE I

TWO WEEKS AVERAGE RATE OF ELONGATION IN TWENTY SELECTED FIRST-ORDER STALKS AND MEAN MAXIMUM AIR TEMPERATURE FOR CORRESPONDING PERIOD

| DATE: TWO WEEKS ENDING | LOW N | MEDIUM N | HIGH N | MEAN MAXIMUM TEMPERATURE | DATE: TWO WEEKS ENDING | LOW N | MEDIUM N | HIGH N | MEAN MAXIMUM TEMPERATURE |
|---|---|---|---|---|---|---|---|---|---|
| | *feet* | *feet* | *feet* | °*F.* | | *feet* | *feet* | *feet* | °*F.* |
| 11/3/33 | 0.68 | 0.68 | 0.84 | 80.7 | 6/1/34 | 0.77 | 0.92 | 0.94 | 81.0 |
| 11/17/33 | 0.56 | 0.48 | 0.54 | 79.2 | 6/15/34 | 0.67 | 0.82 | 0.79 | 82.0 |
| 12/1/33 | 0.45 | 0.58 | 0.46 | 78.5 | 6/29/34 | 0.52 | 0.73 | 0.75 | 81.9 |
| 12/15/33 | 0.41 | 0.54 | 0.46 | 79.4 | 7/13/34 | 0.70 | 0.80 | 0.89 | 83.0 |
| 12/29/33 | 0.55 | 0.54 | 0.65 | 78.4 | 7/27/34 | 0.88 | 0.93 | 0.90 | 83.1 |
| 1/12/34 | 0.56 | 0.51 | 0.46 | 78.6 | 8/10/34 | 0.64 | 0.70 | 0.81 | 83.2 |
| 1/26/34 | 0.50 | 0.53 | 0.58 | 78.1 | 8/24/34 | 0.86 | 0.79 | 0.94 | 83.9 |
| 2/9/34 | 0.33 | 0.40 | 0.40 | 76.1 | 9/7/34 | 0.77 | 0.77 | 0.91 | 84.2 |
| 2/23/34 | 0.27 | 0.29 | 0.35 | 76.4 | 9/21/34 | 0.71 | 0.73 | 1.00 | 84.2 |
| 3/9/34 | 0.41 | 0.45 | 0.56 | 78.6 | 10/5/34 | 0.59 | 0.55 | 0.50 | 83.1 |
| 3/23/34 | 0.44 | 0.46 | 0.46 | 77.7 | 10/19/34 | 0.57 | 0.58 | 0.70 | 83.3 |
| 4/6/34 | 0.31 | 0.36 | 0.36 | 74.1 | 11/2/34 | 0.40 | 0.41 | 0.49 | 82.0 |
| 4/20/34 | 0.21 | 0.29 | 0.37 | 74.4 | 11/16/34 | 0.40 | 0.43 | 0.56 | 80.5 |
| 5/4/34 | 0.30 | 0.44 | 0.46 | 78.6 | 12/7/34 | 0.34 | 0.43 | 0.62 | 80.8 |
| 5/18/34 | 0.50 | 0.60 | 0.63 | 78.1 | 12/28/34 | 0.28 | 0.41 | 0.56 | 80.5 |

series, increasingly so as the season advances. In the medium N series, the growth rate keeps up with that in the high N until the latter part of 1934 when it also tends to lag behind. It is significant that the actual yield of millable cane also begins to show differences due to treatment only after the tenth month. In the high and the medium N series, the cane yields are always very close, as might be expected from the growth data. The data therefore indicate, on the one hand, that the low N series did not suffer from any deficiency of nitrogen until after the tenth month, owing probably to the small requirement of nitrogen under the poor growing conditions of the early period, and, on the other hand, that application of nitrogen in excess of that in the medium series is largely a waste as a growth promoting factor. This should not cause great surprise in view of the fact that the amount applied in the medium N series is known from many field trials to be about the optimum for this variety of cane. It has been found that the seasonal variations in the rate of growth, particularly in the first fifteen months,[4] are quantitatively and linearly related to the changes in temperature. This important relationship will form the subject of another paper. It will be sufficient here to note that such close interdependence of temperature and rate of cane elongation is now a matter of common knowledge from the work of STENDER, DAS, and others cited previously. In view of this temperature relationship, no significance is attached to an apparent influence of the time of application of nitrogen on the growth rate (fig. 2).

We shall refer again to these rate-of-elongation curves, for it will be clearly shown that the seasonal variations in growth rate are significantly related to the seasonal variations in hydration and the variations in the percentage of sucrose, reducing sugars, and other substances in the plant.

RATE OF LEAF AND INTERNODE PRODUCTION.—Figure 3 shows the number of new leaves produced during the two-week periods in the twenty stalks of cane used for length measurements. For the sake of clearness, only the low and the high N series are shown, the medium N series usually occupy a position between the other two. The seasonal variations in leaf production are not so marked as the length variations even though both exhibit considerable parallelism. The treatment differences are, however, clearly brought out. As each leaf denotes an internode, it would appear from this figure that not only the rate of length growth but also the production of internodes is favorably influenced by nitrogen fertilization.

## COMPOSITION AND YIELD OF CROP

COMPOSITION OF CROP.—Table II and figure 4 show the weight of the first- and the second-order stalks harvested periodically as a percentage of

[4] The data after the fifteenth month become increasingly uncertain owing to the death of some stalks and the cessation of top growth in many others as a result of flowering in the fall of 1934.

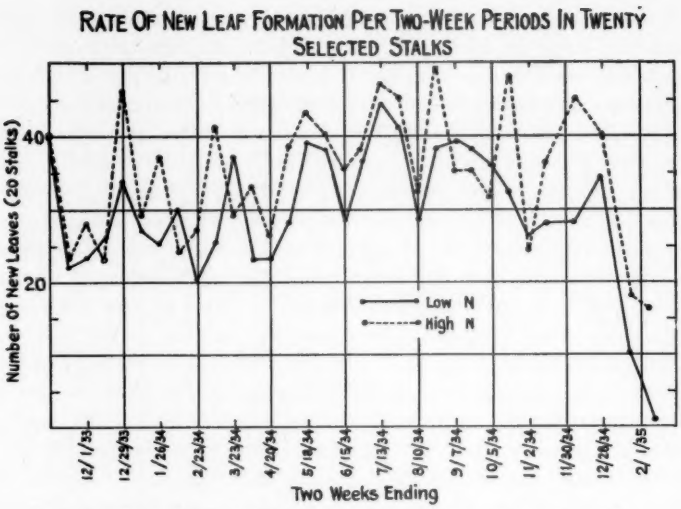



FIG. 3. Rate of new leaf formation in sugar cane, two-week periods.

the total weight of millable cane from all orders of stalks. At six months of age, the entire millable cane weight consists of first-order stalks. From the eighth month on to the twenty-second month, first-order stalks generally constitute about 75 to 80 per cent. of the total weight while the second-order stalks account for about 20 to 25 per cent.; together they total practically 100 per cent. of the entire millable crop. In the high N series, owing to the greater stimulation of tiller growth, the first-order stalks constitute only about 20 per cent. of the millable cane, the second-order a little less than 30 per cent., and the higher orders the remainder. Even in this series, however, the first and second orders together constitute more than 95 per cent. of the total millable cane weight. The relative proportions of the first- and second-order stalks are just the reverse of what was found in a previous study (10), owing no doubt to the fact that in the previous study there was only one mother stalk in each foot length of row as against two and six-tenths in this experiment. A large number of second-order stalks did indeed come up in the present experiment but they were shaded out by the mother stalks. In the previous study it was shown that the number of stalks that reached the millable stage in this variety of cane appeared to be about 2.6 per lineal foot of row; this study shows that by starting originally with that number it may be possible to raise somewhat the average number that can be carried on one foot of soil. This is significant from the practical standpoint.

## TABLE II

MISCELLANEOUS DATA ON YIELDS

| | N | Age (months) at harvest | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8½ | 10½ | 12½ | 14½ | 16½ | 18½ | 20½ | 22½ | 24½ |
| | | Month of harvest | | | | | | | | | | |
| | | Oct. | Jan. | March | May | July | Sept. | Nov. | Jan. | March | May | July |
| First order stalks wt. of millable cane | Low | ..... | 74.3 | 168.8 | 246.4 | 306.3 | 511.5 | 608.6 | 616.0 | 583.8 | 606.4 | 580.5 |
| | Med. | ..... | 59.2 | 130.9 | 259.6 | 407.1 | 637.8 | 743.1 | 775.8 | 851.0 | 726.8 | 634.6 |
| | High | ..... | 84.9 | 178.9 | 263.6 | 388.1 | 619.0 | 734.4 | 706.8 | 698.8 | 542.5 | 397.1 |
| Second order stalks wt. of millable cane | Low | ..... | ..... | 38.7 | 62.6 | 65.7 | 135.0 | 156.0 | 164.0 | 179.8 | 169.5 | 253.6 |
| | Med. | ..... | ..... | 37.3 | 97.1 | 140.6 | 130.3 | 223.5 | 260.8 | 249.0 | 190.9 | 241.4 |
| | High | ..... | ..... | 46.8 | 94.4 | 162.0 | 205.1 | 358.8 | 285.5 | 254.0 | 363.4 | 229.1 |
| All orders combined wt. of millable cane | Low | ..... | 74.3 | 204.5 | 321.0 | 372.0 | 669.5 | 771.1 | 787.0 | 782.0 | 800.9 | ..... |
| | Med. | ..... | 59.2 | 168.5 | 370.7 | 541.7 | 771.5 | 987.6 | 1062.5 | 1116.0 | 936.7 | ..... |
| | High | ..... | 84.9 | 227.6 | 369.0 | 585.1 | 856.0 | 1103.2 | 1011.8 | 971.8 | 941.9 | ..... |
| All orders combined wt. of millable cane & non-mill. top | Low | 75.3 | 223.3 | 398.3 | 462.5 | 560.9 | 867.3 | 938.3 | 891.0 | 908.8 | 926.7 | ..... |
| | Med. | 59.3 | 215.8 | 363.7 | 539.1 | 812.9 | 969.3 | 1182.8 | 1196.5 | 1241.0 | 1057.1 | ..... |
| | High | 65.9 | 258.5 | 467.8 | 556.9 | 834.2 | 1070.3 | 1254.2 | 1114.8 | 1093.3 | 1080.0 | ..... |
| Percentage N recovered in plants as % of total amount applied | Low | 127.4 | 148.8 | 160.0 | 104.4 | 92.4 | 110.90 | 103.2 | 105.1 | 75.9 | 81.3 | ..... |
| | Med. | 49.7 | 73.5 | 75.5 | 66.9 | 75.3 | 79.0 | 72.0 | 77.5 | 62.3 | 51.4 | ..... |
| | High | 38.7 | 55.2 | 49.2 | 47.0 | 43.3 | 36.0 | 42.2 | 36.2 | 25.4 | 25.8 | ..... |
| Ratio of millable dry-leaf to millable green-leaf cane | Low | ..... | 0.56 | 1.40 | 2.91 | 3.82 | 2.50 | 4.90 | 6.51 | 8.73 | 10.43 | ..... |
| | Med. | ..... | 0.47 | 1.04 | 3.05 | 3.12 | 3.19 | 5.33 | 7.64 | 15.70 | 12.21 | ..... |
| | High | ..... | 0.58 | 1.29 | 2.43 | 2.58 | 2.83 | 7.40 | 12.92 | 10.45 | 13.66 | ..... |

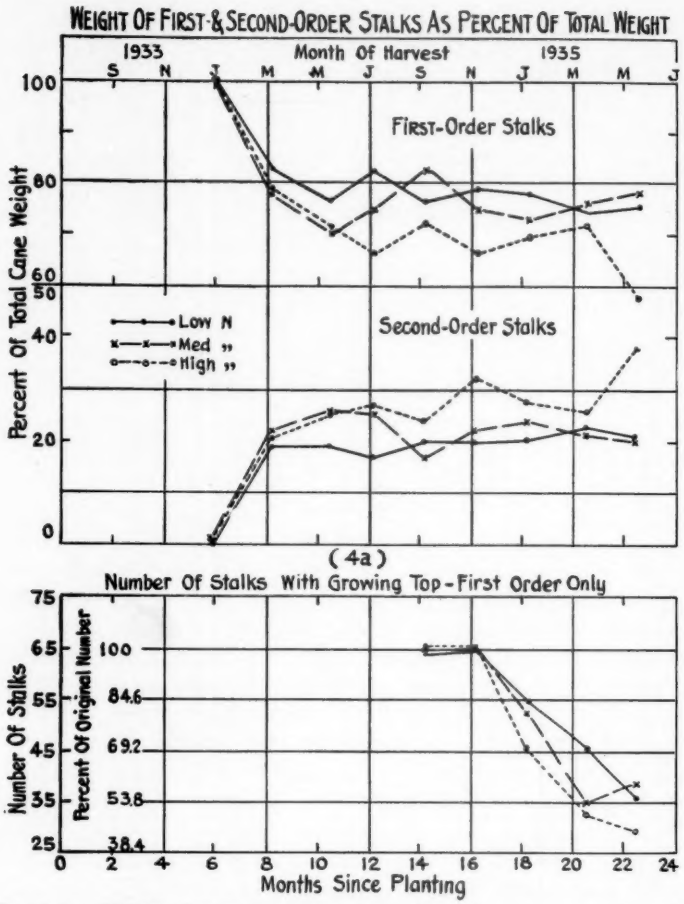



FIG. 4–4a. Weight of first- and second-order stalks of sugar cane as percentage of total weight. 4a. Number of stalks with growing tops (first-order only) with increasing age.

STALK MORTALITY.—Figure 4 *a* shows the number of growing stalks, *i.e.*, stalks with a growing top, harvested at different periods. Up to the sixteenth month practically all the stalks had growing tops. Later the number declined rapidly, not only in the high but also in the low and the medium N series. This no doubt indicates that the trend of the decline was not initiated by a harmful excess of nitrogen. The differential rate of decline is probably connected with differential fertilization.

Part of this decline is accounted for by the stalks that tasseled and in consequence ceased vegetative growth; but for the greater part the decline is due to stalks that gradually weakened and started to die off. When expressed as percentage of the original number, the rate of mortality appears to be roughly 15 per cent. every two months. Should the decline continue, then one would expect to harvest practically no first-order stalks at about thirty months of age. Rather significantly, this rate of decline is about the same as was found in the previous study referred to earlier. The stalk mortality must therefore be in some way related to the nature of the plant itself.

YIELD OF CANE IN FIRST-ORDER STALKS.—Tables III, IV, and V (section A) and figure 5 show the fresh weight per row of the top, the millable green-leaf and the dry-leaf cane harvested at various ages. In the case of the top and the green-leaf sections, each harvest represents entirely new growth and as such the yield curves are "rate-of-production" curves. In the dry-leaf section only the accumulated effect is observed.

As might have been expected, the green weight of tops follows in general the different levels of fertilizations. When after the fourteenth month the production in the high N series falls off owing to mounting top mortality, the medium N series becomes the heaviest producer. In the later months all the series seem to be more or less alike. The seasonal variations in the weight of top parallel in general the changes in the rate of elongation.

In the green-leaf section the differences due to treatment are even more marked than in the top. Here also the yield is generally greater the greater the N application. Again the yield in the high N series falls off after the fourteenth month and all the series approach each other after the twentieth month. The great increase in weight shown in the September harvest strikingly supports the growth curves in figure 2.

In the dry-leaf section, all the series yield practically the same up to the tenth month and from then on the yield curves increasingly diverge. It will be recalled that the growth curves also begin to separate at about this time. The low N series begins to flatten out after the sixteenth month, unquestionably reflecting the lack of nitrogen. The high N series does the same at the same time, but owing, as we have seen, to the increasing mortality of the tops as a result of too much nitrogen. The medium N series shows a steady increase in yield up to the twentieth month. Then there follows a rapid decline as the stalks begin to die. For the most part, however, the high and the medium N series yield practically the same, a result which might well have been predicted from the rate-of-growth data.

TOTAL YIELD OF FRESH CANE FROM ALL ORDERS OF STALKS.—Figure 6 (data in tables III, IV, V) shows the total yield per row of millable cane and

## TABLE III

FIRST-ORDER STALKS: NON-MILLABLE TOP

| | N | Age (months) at harvest | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8½ | 10½ | 12½ | 14½ | 16½ | 18½ | 20½ | 22½ | 24½ |
| | | Month of harvest | | | | | | | | | | |
| | | Oct. | Jan. | March | May | July | Sept. | Nov. | Jan. | March | May | July |
| A. Green weight of cane per row in pounds | Low | 75.3 | 102.69 | 101.50 | 77.38 | 108.40 | 99.5 | 81.3 | 47.0 | 53.0 | 35.8 | 34.1 |
| | Med. | 59.3 | 86.00 | 92.44 | 88.75 | 125.5 | 116.12 | 103.0 | 54.3 | 36.0 | 33.8 | 28.4 |
| | High | 65.9 | 111.63 | 108.94 | 103.10 | 124.8 | 115.75 | 66.0 | 37.0 | 35.0 | 29.5 | 21.4 |
| B. Percentage dry matter on green weight | Low | 17.70 | 19.82 | 19.61 | 20.41 | 21.10 | 20.63 | 21.27 | 25.59 | 22.28 | 24.68 | 26.35 |
| | Med. | 17.00 | 18.24 | 18.53 | 19.19 | 20.41 | 16.89 | 19.77 | 35.50 | 21.02 | 24.87 | 23.61 |
| | High | 17.25 | 17.25 | 17.13 | 17.43 | 18.11 | 15.60 | 20.12 | 30.33 | 21.00 | 26.24 | 27.92 |
| C. Grams of water per gram dry matter | Low | 4.64 | 4.04 | 4.09 | 3.89 | 3.73 | 3.84 | 3.70 | 2.90 | 3.48 | 3.05 | 2.19 |
| | Med. | 4.88 | 4.48 | 4.39 | 4.21 | 3.89 | 4.92 | 4.05 | 1.81 | 3.75 | 3.02 | 3.24 |
| | High | 4.19 | 4.79 | 4.83 | 4.73 | 4.52 | 5.41 | 3.97 | 2.29 | 3.76 | 2.81 | 2.58 |
| D. Percentage sucrose in dry matter | Low | 5.99 | 4.81 | 3.60 | 6.39 | 6.47 | 2.41 | 3.05 | 5.15 | 5.46 | 5.16 | ..... |
| | Med. | 8.58 | 4.02 | 5.69 | 6.08 | 6.23 | 3.71 | 2.82 | 1.41 | 4.09 | 5.46 | ..... |
| | High | ..... | 4.99 | 5.22 | 6.14 | 4.24 | 3.57 | 3.36 | 2.17 | 8.86 | 5.84 | ..... |
| E. Percentage red. sugars in dry matter | Low | 3.98 | 3.91 | 1.95 | 3.32 | 4.60 | 5.90 | 4.01 | 1.50 | 1.93 | 2.54 | ..... |
| | Med. | 3.55 | 4.27 | 2.30 | 4.35 | 5.32 | 7.73 | 4.26 | 0.97 | 2.72 | 2.69 | ..... |
| | High | ..... | 5.65 | 2.62 | 5.48 | 6.72 | 8.38 | 4.54 | 2.01 | 2.81 | 2.53 | ..... |
| F. Percentage polysaccharides in dry matter | Low | 15.99 | 19.89 | 22.14 | 24.60 | 25.14 | 20.82 | 23.28 | 23.37 | 27.91 | 23.13 | ..... |
| | Med. | 21.15 | 20.78 | 20.16 | 26.03 | 24.74 | 20.12 | 24.62 | 9.06 | 22.73 | 21.43 | ..... |
| | High | 20.43 | 20.02 | 19.06 | 25.04 | 25.69 | 26.11 | 24.29 | 18.35 | 16.04 | 21.29 | ..... |
| G. Percentge total N in dry matter | Low | 0.686 | 0.704 | 0.841 | 0.749 | 0.630 | 0.787 | 0.851 | 0.979 | 0.697 | 0.657 | ..... |
| | Med. | 0.707 | 0.708 | 0.908 | 0.862 | 0.791 | 0.929 | 0.799 | 0.887 | 0.910 | 0.770 | ..... |
| | High | 0.735 | 0.749 | 0.983 | 0.987 | 1.106 | 0.853 | 0.956 | 1.051 | 0.800 | 0.884 | ..... |

TABLE III.—(*Continued*)

| | N | Age (months) at harvest | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8½ | 10½ | 12½ | 14½ | 16½ | 18½ | 20½ | 22½ | 24½ |
| | | Month of harvest | | | | | | | | | | |
| | | Oct. | Jan. | March | May | July | Sept. | Nov. | Jan. | March | May | July |
| H. Percentage alc. soluble N in dry matter | Low | 0.061 | 0.056 | 0.068 | 0.066 | 0.077 | 0.0607 | 0.068 | 0.110 | 0.055 | 0.074 | ..... |
| | Med. | 0.094 | 0.063 | 0.078 | 0.075 | 0.071 | 0.0817 | 0.070 | 0.053 | 0.071 | 0.071 | ..... |
| | High | 0.092 | 0.080 | 0.102 | 0.112 | 0.125 | 0.086 | 0.122 | 0.070 | 0.074 | 0.100 | ..... |
| I. Percentage sucrose in expressed juice | Low | 3.24 | 2.23 | 2.04 | 2.16 | 2.28 | 3.20 | 2.08 | 1.97 | 1.41 | 2.38 | 2.71 |
| | Med. | 3.21 | 1.59 | 1.91 | 1.50 | 1.51 | .....* | 1.66 | 1.54 | 1.72 | 1.60 | 3.01 |
| | High | 3.58 | 1.55 | 1.53 | 0.89 | 0.97 | .....* | 0.92 | 0.89 | 1.23 | 1.94 | 2.90 |
| J. Percentage red. sugars in expressed juice | Low | 2.36 | 2.85 | 2.53 | 1.99 | 3.29 | 3.10 | 2.41 | 1.97 | 1.39 | 1.69 | 3.38 |
| | Med. | 2.24 | 2.83 | 2.76 | 2.69 | 3.61 | 3.58 | 2.68 | 1.79 | 1.52 | 2.07 | 3.06 |
| | High | 2.40 | 2.37 | 2.74 | 2.57 | 3.40 | 3.65 | 2.58 | 2.19 | 2.32 | 2.47 | 2.59 |
| K. Conductivity of expressed juice ($\times 10^3$) | Low | 13.2 | 25.45 | 13.21 | 14.66 | 11.57 | 9.44 | 11.21 | 18.21 | 18.79 | 15.06 | 12.52 |
| | Med. | 14.3 | 32.95 | 13.20 | 13.66 | 10.51 | 9.12 | 10.43 | 18.56 | 20.96 | 16.69 | 14.18 |
| | High | 13.2 | 33.15 | 12.15 | 12.90 | 11.33 | 8.55 | 11.00 | 19.10 | 18.29 | 15.16 | 13.31 |
| L. pH of expressed juice | Low | 4.79 | 5.04 | 4.88 | 4.96 | 4.94 | 4.73 | 5.59 | 5.23 | 4.95 | 5.17 | 5.04 |
| | Med. | 4.79 | 4.96 | 4.92 | 4.96 | 4.94 | 4.88 | 4.91 | 5.21 | 5.09 | 5.00 | 5.13 |
| | High | 4.79 | 4.96 | 4.88 | 4.90 | 5.00 | 4.90 | 4.66 | 5.00 | 4.91 | 5.05 | 4.95 |
| M. Ratio $\frac{\text{Total N}}{\text{Soluble N}}$ | Low | 11.2 | 12.6 | 12.4 | 11.3 | 8.2 | 13.0 | 12.5 | 8.9 | 11.9 | 8.9 | ..... |
| | Med. | 7.5 | 11.2 | 11.6 | 11.5 | 11.1 | 11.4 | 11.4 | 16.7 | 12.8 | 10.8 | ..... |
| | High | 8.0 | 9.4 | 9.6 | 8.8 | 8.8 | 9.9 | 7.8 | 15.0 (†) | 10.8 | 8.8 | ..... |

* Negative polariscope reading.

## TABLE IV

FIRST-ORDER STALKS: MILLABLE GREEN-LEAF SECTION

| | N | AGE (MONTHS) AT HARVEST | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8¼ | 10½ | 12¼ | 14¼ | 16¼ | 18¼ | 20½ | 22½ | 24¼ |
| | | DATE OF HARVEST | | | | | | | | | | |
| | | OCT. | JAN. | MARCH | MAY | JULY | SEPT. | NOV. | JAN. | MARCH | MAY | JULY |
| A. Green weight of cane per row in pounds | Low | ..... | 47.44 | 70.25 | 63.00 | 63.50 | 146.0 | 103.12 | 82.0 | 60.0 | 53.0 | 44.9 |
| | Med. | ..... | 40.44 | 64.25 | 64.00 | 98.80 | 150.25 | 117.12 | 89.75 | 51.0 | 55.0 | 31.8 |
| | High | ..... | 53.94 | 78.57 | 76.75 | 108.40 | 161.50 | 87.4 | 50.75 | 61.0 | 37.0 | 28.3 |
| B. Percentage dry matter on green weight | Low | ..... | 19.29 | 21.20 | 23.47 | 23.29 | 19.31 | 21.35 | 20.25 | 25.32 | 25.91 | 25.14 |
| | Med. | ..... | 20.88 | 20.40 | 23.06 | 18.79 | 16.78 | 20.12 | 24.07 | 23.08 | 26.61 | 26.17 |
| | High | ..... | 18.22 | 17.48 | 21.63 | 17.95 | 20.85 | 19.18 | 22.14 | 26.11 | 25.74 | 26.89 |
| C. Grams of water per gram dry matter | Low | ..... | 4.18 | 3.71 | 3.26 | 3.29 | 4.17 | 3.68 | 3.93 | 2.94 | 2.85 | 2.98 |
| | Med. | ..... | 3.78 | 3.90 | 3.33 | 4.35 | 4.95 | 3.97 | 3.47 | 3.33 | 2.75 | 2.82 |
| | High | ..... | 4.48 | 4.72 | 3.62 | 4.57 | 3.79 | 4.21 | 3.51 | 2.82 | 2.88 | 2.76 |
| D. Percentage sucrose in dry matter | Low | ..... | 31.24 | 38.74 | 41.50 | 41.79 | 29.53 | 36.39 | 52.29 | 43.46 | 37.45 | ..... |
| | Med. | ..... | 28.94 | 38.24 | 43.42 | 37.18 | 34.52 | 39.44 | 48.64 | 52.09 | 43.70 | ..... |
| | High | ..... | 32.82 | 36.00 | 43.46 | 31.59 | 26.68 | 35.23 | 42.35 | 47.83 | 35.19 | ..... |
| E. Percentage red. sugars in dry matter | Low | ..... | 9.36 | 4.28 | 4.34 | 5.19 | 10.17 | 7.91 | 4.96 | 1.97 | 2.88 | ..... |
| | Med. | ..... | 8.76 | 4.90 | 4.92 | 9.23 | 11.26 | 10.48 | 2.62 | 2.59 | 3.38 | ..... |
| | High | ..... | 11.26 | 4.06 | 6.56 | 14.07 | 15.26 | 14.37 | 5.96 | 1.86 | 2.50 | ..... |
| F. Percentage polysaccharides in dry matter | Low | ..... | 16.27 | 11.16 | 12.20 | 15.07 | 13.15 | 13.57 | 14.67 | 14.21 | 15.63 | ..... |
| | Med. | ..... | 15.83 | 11.54 | 12.70 | 15.08 | 13.25 | 11.08 | 13.71 | 11.88 | 15.63 | ..... |
| | High | ..... | 16.12 | 10.17 | 12.06 | 14.14 | 13.17 | 12.00 | 13.93 | 13.02 | 16.91 | ..... |
| G. Percentage total N in dry matter | Low | ..... | 0.142 | 0.190 | 0.145 | 0.101 | 0.131 | 0.189 | 0.165 | 0.167 | 10.204 | ..... |
| | Med. | ..... | 0.186 | 0.161 | 0.147 | 0.195 | 0.209 | 0.165 | 0.185 | 0.268 | 0.224 | ..... |
| | High | ..... | 0.180 | 0.229 | 0.207 | 0.204 | 0.213 | 0.248 | 0.271 | 0.238 | 0.349 | ..... |

TABLE IV.—(*Continued*)

| | N | Age (months) at harvest | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8½ | 10½ | 12½ | 14½ | 16½ | 18½ | 20½ | 22½ | 24½ |
| | | Date of harvest | | | | | | | | | | |
| | | Oct. | Jan. | March | May | July | Sept. | Nov. | Jan. | March | May | July |
| H. Percentage alc. soluble N in dry matter | Low | ..... | 0.037 | 0.050 | 0.032 | 0.031 | 0.0326 | 0.064 | 0.070 | 0.045 | 0.049 | ..... |
| | Med. | ..... | 0.030 | 0.060 | 0.045 | 0.065 | 0.0989 | 0.078 | 0.054 | 0.088 | 0.068 | ..... |
| | High | ..... | 0.035 | 0.078 | 0.132 | 0.080 | 0.091 | 0.128 | 0.095 | 0.068 | 0.111 | ..... |
| I. Percentage sucrose in expressed juice | Low | ..... | 10.31 | 12.55 | 13.43 | 11.56 | 9.69 | 11.13 | 13.91 | 16.60 | 17.42 | ..... |
| | Med. | ..... | 8.44 | 11.55 | 12.00 | 9.83 | 7.27 | 9.96 | 14.17 | 15.27 | 14.78 | ..... |
| | High | ..... | 7.70 | 9.42 | 10.22 | 6.96 | 5.52 | 7.77 | 10.56 | 14.88 | 16.08 | ..... |
| J. Percentage red. sugars in expressed juice | Low | ..... | 2.46 | 1.73 | 1.24 | 1.74 | 2.80 | 2.14 | 1.08 | 0.34 | 0.35 | ..... |
| | Med. | ..... | 2.90 | 1.85 | 1.81 | 2.32 | 3.20 | 2.67 | 1.00 | 0.57 | 0.86 | ..... |
| | High | ..... | 3.10 | 2.43 | 2.40 | 3.12 | 3.88 | 3.07 | 2.20 | 0.50 | 0.80 | ..... |
| K. Conductivity of expressed juice ($\times 10^3$) | Low | ..... | 4.13 | 3.96 | 3.93 | 3.63 | 4.39 | 5.11 | 5.57 | 4.19 | 3.42 | ..... |
| | Med. | ..... | 6.29 | 5.15 | 4.13 | 3.75 | 4.58 | 4.44 | 5.09 | 5.31 | 3.57 | ..... |
| | High | ..... | 11.33 | 5.49 | 4.41 | 4.05 | 6.64 | 4.47 | 5.38 | 4.57 | 3.00 | ..... |
| L. pH of expressed juice | Low | ..... | 5.41 | 5.37 | 5.33 | 5.36 | 5.20 | 5.27 | 5.16 | 5.21 | 5.29 | ..... |
| | Med. | ..... | 5.41 | 5.37 | 5.37 | 5.29 | 5.30 | 5.23 | 5.27 | 5.17 | 5.22 | ..... |
| | High | ..... | 5.41 | 5.37 | 5.30 | 5.29 | 5.25 | 5.10 | 5.23 | 5.23 | 5.28 | ..... |
| M. Cane ratio of expressed juice | Low | ..... | 15.33 | 11.93 | 10.70 | 13.09 | 17.68 | 13.92 | 10.20 | 8.07 | 7.60 | ..... |
| | Med. | ..... | 22.99 | 13.44 | 12.51 | 16.82 | 20.16 | 16.97 | 10.30 | 8.95 | 9.33 | ..... |
| | High | ..... | 27.20 | 18.58 | 16.57 | 32.39 | 62.99 | 27.69 | 15.25 | 9.38 | 8.45 | ..... |
| N. Ratio $\frac{\text{Total N}}{\text{Soluble N}}$ | Low | ..... | 3.8 | 3.8 | 4.5 | 3.3 | 4.0 | 3.0 | 2.4 | 3.7 | 4.2 | ..... |
| | Med. | ..... | 6.2 | 2.7 | 3.3 | 3.0 | 2.1 | 2.1 | 3.4 | 3.0 | 3.3 | ..... |
| | High | ..... | 5.1 | 3.1 | 1.6 | 2.6 | 2.3 | 1.9 | 2.9 | 3.5 | 3.1 | ..... |

## TABLE V

FIRST-ORDER STALKS: MILLABLE DRY-LEAF SECTION

| | N | AGE (MONTHS) AT HARVEST | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8½ | 10½ | 12½ | 14½ | 16½ | 18½ | 20½ | 22½ | 24½ |
| | | DATE OF HARVEST | | | | | | | | | | |
| | | OCT. | JAN. | MARCH | MAY | JULY | SEPT. | NOV. | JAN. | MARCH | MAY | JULY |
| A. Green weight of cane per row in pounds | Low | ..... | 26.88 | 98.50 | 183.44 | 242.80 | 365.5 | 505.5 | 534.0 | 524.0 | 553.0 | 535.6 |
| | Med. | ..... | 19.19 | 66.94 | 195.63 | 208.30 | 487.5 | 626.0 | 686.0 | 801.0 | 672.0 | 602.9 |
| | High | ..... | 31.00 | 102.10 | 186.80 | 279.70 | 475.5 | 647.0 | 656.0 | 638.0 | 505.5 | 368.8 |
| B. Percentage dry matter on green weight | Low | ..... | 25.46 | 25.03 | 27.10 | 30.58 | 28.10 | 26.68 | 24.80 | 28.15 | 29.47 | 30.57 |
| | Med. | ..... | 24.98 | 24.43 | 25.40 | 27.71 | 22.73 | 24.35 | 26.81 | 27.06 | 28.95 | 30.02 |
| | High | ..... | 24.07 | 22.32 | 25.29 | 24.90 | 23.39 | 21.49 | 21.56 | 26.28 | 26.96 | 27.88 |
| C. Grams of water per gram dry matter | Low | ..... | 2.92 | 2.99 | 2.69 | 2.27 | 2.55 | 2.89 | 3.03 | 2.55 | 2.39 | 2.33 |
| | Med. | ..... | 3.00 | 3.09 | 2.93 | 2.61 | 3.39 | 3.10 | 2.72 | 2.69 | 2.45 | 2.27 |
| | High | ..... | 3.15 | 3.48 | 2.95 | 3.01 | 3.27 | 3.65 | 3.63 | 2.80 | 2.70 | 2.59 |
| D. Percentage sucrose in dry matter | Low | ..... | 32.51 | 38.91 | 41.85 | 46.23 | 40.67 | 42.03 | 47.50 | 50.16 | 39.79 | ..... |
| | Med. | ..... | 29.63 | 41.08 | 47.69 | 49.62 | 33.35 | 40.74 | 52.19 | 51.60 | 48.64 | ..... |
| | High | ..... | 32.86 | 38.28 | 44.48 | 48.34 | 36.14 | 39.44 | 46.70 | 50.28 | 49.77 | ..... |
| E. Percentage red. sugars in dry matter | Low | ..... | 2.32 | 2.01 | 1.25 | 1.03 | 1.71 | 3.48 | 2.29 | 0.64 | 2.00 | ..... |
| | Med. | ..... | 2.52 | 2.20 | 1.64 | 1.46 | 3.92 | 4.67 | 1.54 | 1.38 | 2.33 | ..... |
| | High | ..... | 3.67 | 2.90 | 2.17 | 2.39 | 3.79 | 6.33 | 4.91 | 1.36 | 2.42 | ..... |
| F. Percentage polysaccharides in dry matter | Low | ..... | 16.17 | 11.50 | 13.12 | 13.22 | 12.83 | 13.25 | 12.44 | 11.31 | 12.68 | ..... |
| | Med. | ..... | 18.87 | 10.95 | 13.23 | 13.58 | 13.30 | 12.07 | 10.76 | 10.80 | 10.93 | ..... |
| | High | ..... | 15.50 | 11.61 | 13.41 | 11.48 | 12.82 | 13.01 | 12.83 | 11.61 | 11.44 | ..... |
| G. Percentage total N in dry matter | Low | ..... | 0.160 | 0.110 | 0.093 | 0.069 | 0.079 | 0.114 | 0.126 | 0.141 | 0.0895 | ..... |
| | Med. | ..... | 0.201 | 0.130 | 0.101 | 0.084 | 0.184 | 0.158 | 0.1258 | 0.122 | 0.0985 | ..... |
| | High | ..... | 0.209 | 0.160 | 0.196 | 0.202 | 0.208 | 0.253 | 0.217 | 0.142 | 0.1380 | ..... |

TABLE V.—(*Continued*)

FIRST-ORDER STALKS: MILLABLE DRY-LEAF SECTION

| | N | Age (months) at harvest | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3½ | 6 | 8¼ | 10½ | 12¼ | 14¼ | 16¼ | 18¼ | 20½ | 22½ | 24¼ |
| | | Date of harvest | | | | | | | | | | |
| | | Oct. | Jan. | March | May | July | Sept. | Nov. | Jan. | March | May | July |
| H. Percentage alc. soluble N in dry matter | Low | ..... | 0.033 | 0.024 | 0.029 | 0.019 | 0.035 | 0.024 | 0.027 | 0.023 | 0.024 | ..... |
| | Med. | ..... | 0.062 | 0.044 | 0.024 | 0.027 | 0.095 | 0.064 | 0.032 | 0.037 | 0.028 | ..... |
| | High | ..... | 0.067 | 0.094 | 0.106 | 0.114 | 0.1212 | 0.1360 | 0.1040 | 0.047 | 0.058 | ..... |
| I. Percentage sucrose in expresed juice | Low | ..... | 14.95 | 17.05 | 18.56 | 18.45 | 15.96 | 15.17 | 14.46 | 16.93 | 18.21 | ..... |
| | Med. | ..... | 13.91 | 15.45 | 17.57 | 17.65 | 12.35 | 13.67 | 16.85 | 17.16 | 17.90 | ..... |
| | High | ..... | 13.19 | 14.14 | 14.58 | 14.64 | 10.35 | 8.31 | 11.54 | 15.44 | 15.42 | ..... |
| J. Percentage of red. sugars in expressed juice | Low | ..... | 0.74 | 0.47 | 0.20 | 0.28 | 0.66 | 0.69 | 0.67 | 0.37 | 0.28 | ..... |
| | Med. | ..... | 1.11 | 0.77 | 0.52 | 0.45 | 2.58 | 1.13 | 0.49 | 0.40 | 0.44 | ..... |
| | High | ..... | 1.36 | 0.99 | 0.64 | 0.81 | 1.64 | 1.97 | 1.33 | 0.53 | 0.67 | ..... |
| K. Conductivity of expressed juice ($\times 10^3$) | Low | ..... | 3.10 | 1.87 | 1.98 | 1.98 | 2.39 | 2.88 | 4.04 | 3.65 | 3.04 | ..... |
| | Med. | ..... | 3.57 | 2.54 | 1.69 | 2.09 | 2.98 | 3.01 | 3.24 | 3.77 | 2.27 | ..... |
| | High | ..... | 3.35 | 3.01 | 2.86 | 2.45 | 3.08 | 3.88 | 4.27 | 4.06 | 3.47 | ..... |
| L. pH of expressed juice | Low | ..... | 5.50 | 5.54 | 5.46 | 5.46 | 5.31 | 5.53 | 5.23 | 5.25 | 5.32 | ..... |
| | Med. | ..... | 5.55 | 5.46 | 5.53 | 5.42 | 5.40 | 5.86 | 5.37 | 5.38 | 4.95 | ..... |
| | High | ..... | 5.50 | 5.54 | 5.53 | 5.42 | 5.40 | 5.25 | 5.04 | 5.29 | 5.20 | ..... |
| M. Cane ratio of expressed juice | Low | ..... | 8.85 | 7.77 | 6.97 | 6.98 | 8.32 | 8.81 | 9.35 | 7.78 | 7.25 | ..... |
| | Med. | ..... | 9.90 | 8.79 | 7.45 | 7.41 | 11.37 | 10.05 | 8.07 | 7.73 | 7.35 | ..... |
| | High | ..... | 10.74 | 9.86 | 9.39 | 9.19 | 14.33 | 19.62 | 12.40 | 8.61 | 8.72 | ..... |
| N. Ratio $\frac{\text{Total N}}{\text{Soluble N}}$ | Low | ..... | 4.9 | 4.6 | 3.2 | 3.6 | 2.3 | 4.8 | 4.7 | 6.1 | 3.7 | ..... |
| | Med. | ..... | 3.2 | 3.0 | 4.2 | 3.1 | 2.9 | 2.5 | 4.0 | 3.3 | 3.5 | ..... |
| | High | ..... | 3.1 | 1.7 | 1.9 | 1.8 | 1.7 | 1.9 | 2.1 | 3.0 | 2.4 | ..... |

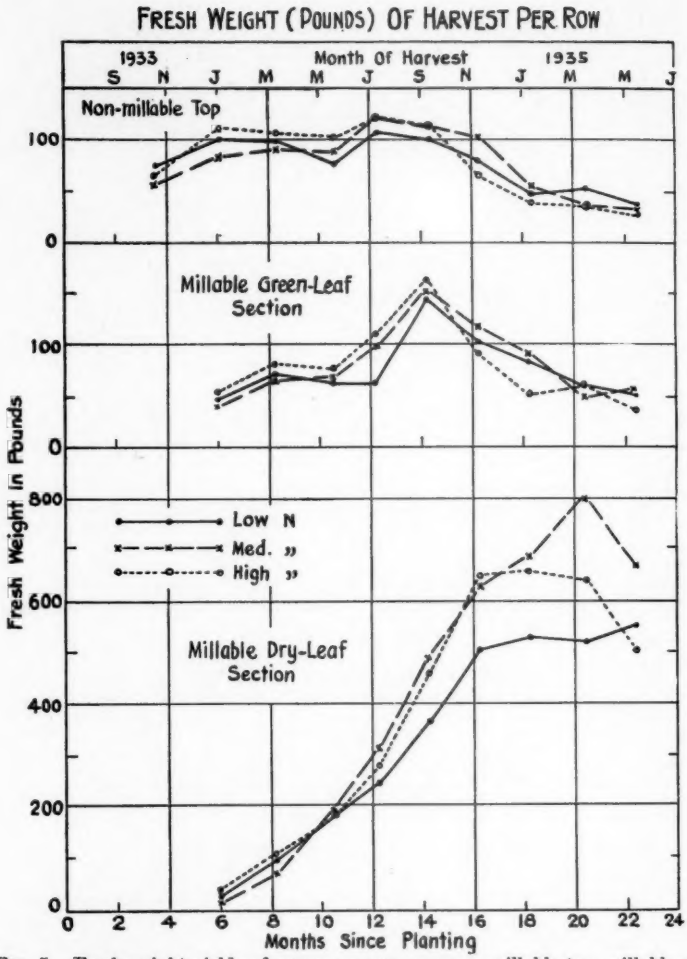



FIG. 5. Fresh weight yields of sugar cane per row, non-millable top, millable green-leaf section, and millable dry-leaf section, with low, medium, and high N.

millable cane plus top from all orders of stalks in the three treatments. Here the progress of yield from harvest to harvest is essentially the same as was noted in the first-order stalks; this is to be expected in view of the fact that these constitute 70 to 80 per cent. of the total millable cane. In the case of the total yield, however, the high N series is usually slightly better than the medium N series, the reverse of what was found in the first-

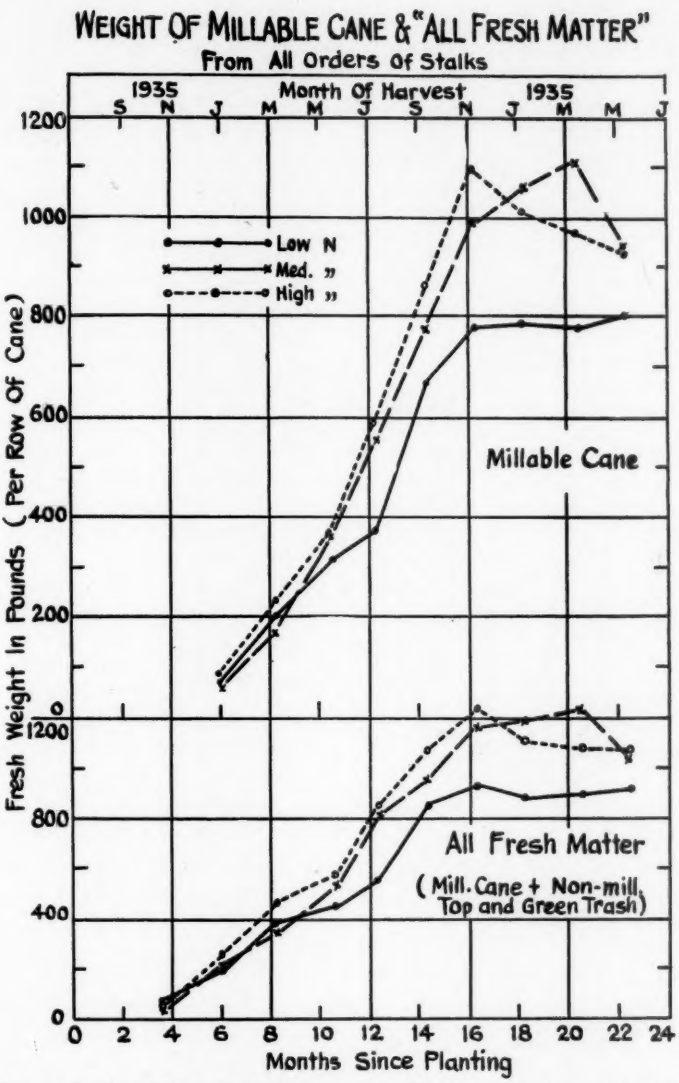

WEIGHT OF MILLABLE CANE & "ALL FRESH MATTER"
From All Orders Of Stalks
1935
Month Of Harvest
1935
S N J M M J S N J M M J
Low N
Med. "
High "
Millable Cane
All Fresh Matter
( Mill. Cane + Non-mill. Top and Green Trash)
Fresh Weight In Pounds ( Per Row Of Cane)
0 200 400 600 800 1000 1200
0 2 4 6 8 10 12 14 16 18 20 22 24
Months Since Planting

FIG. 6. Fresh weight total yield per row of millable cane, and of all fresh matter, with low, medium, and high N fertilization.

order stalks. This result is naturally explained by the presence of a relatively greater proportion of stalks of higher orders in the high N than in the other two series.

RATIO OF DRY-LEAF TO GREEN-LEAF PART.—A point of practical importance is the relative proportions of the green-leaf and the dry-leaf parts in millable cane. As the quality (*i.e.*, percentage sucrose in juice) of the whole cane is an average of the quality in these two parts, and as the green-leaf section under conditions of active growth is always very poor in quality, a high ratio is preferred in the cane that is going to be harvested. The poor quality of young cane is no doubt due largely to the relatively small ratio of the dry-leaf to the green-leaf section. It has often been suggested that a simple physical measurement of this ratio will be as useful an index of maturity or quality in cane as would an actual analysis of juice. Table I shows the increase in this ratio with age of the cane. Interesting differences are to be observed in the three series of treatments.

FLOWERING OF CANE.—The record of tasseling supports the general belief that heavy applications of nitrogen inhibit flowering in cane. The data of the last three harvests show the total number of first-order stalks that tasseled to be 47, 47, and 27 respectively in the low, the medium, and the high N series. There is also some indication that in the high N series tasseling was considerably delayed beyond the other two treatments.

SECOND-ORDER STALKS.—The data on this order are so similar to those of the first-order stalks, that no attempt has been made to include them in this paper or to discuss them separately.

## Physico-chemistry

### CHEMICAL METHODS

SAMPLING.—The samples of cane for chemical analysis consisted, as has previously been stated, of five first-order stalks taken at intervals of 5 feet in the row and about two or three second-order stalks from the immediate vicinity of the mother stalks. The plants were always cut early in the morning, immediately taken to the laboratory, the different plant parts separated, and the gross fresh weights recorded. The tissue was then minced with a slicer which cut uniform sections about one-eighth of an inch thick. Quadruplicate samples of tissue were weighed. Two of these were used for dry weight and total N determinations. The other two were at once placed in boiling 95 per cent. alcohol and boiled for several minutes with a little calcium carbonate added to neutralize acidity. The mixture was cooled and stored in Mason jars for several weeks before being used for analysis.

DRY WEIGHT.—Twenty-five to 50 gm. of tissue, depending on whether from the top or the millable cane, were weighed into numbered aluminum soil tins. These were placed in an electric oven at about 80° C. Usually a constant weight was reached after forty-eight hours. With all possible

speed, the tissue could not be weighed and placed in the oven in less than three hours after cutting. This delay might have caused some error in the moisture determination of the leaf tissue.

TOTAL NITROGEN.—Total nitrogen was determined on the ovendry material using the official Kjeldahl procedure modified to include nitrates.

ALCOHOL-SOLUBLE CONSTITUENTS.—The tissue preserved in alcohol was passed through a household meat grinder, extracted with 80 per cent. alcohol, and filtered. The residue was then twice ground in a mortar using pure silica sand to help disrupt the tissue, and each time extracted with more 80 per cent. alcohol. The extract was then made up to a volume of 500 cc. with 80 per cent. alcohol. Aliquots were taken for the various determinations.

SOLUBLE NITROGEN.—Two hundred and fifty cc. of the alcohol extract were evaporated to dryness under vacuum as suggested by RANKER (**26**) and total N determined on the residue.

REDUCING SUGARS.—Twenty-five cc. of the alcohol extract were evaporated to dryness on a water bath. The residue was then dissolved in water, treated with basic lead acetate and filtered, and the excess lead removed by potassium dihydrogen phosphate. The filtrate from this was made to different volumes, depending on the concentration of sugars as determined by a preliminary run. The reducing sugars were determined by the iodometric method of SHAFFER and HARTMANN (**29**).

SUCROSE.—Sucrose was calculated as the difference between the total and the reducing sugars, and is expressed as reducing sugar. Total sugar was determined by the same iodometric methods after inverting the sugar solution with 0.5 per cent. HCl at a temperature of 65° C. for 30 minutes.

EASILY HYDROLYZABLE POLYSACCHARIDES.—The finely divided residue from the alcohol extraction was evaporated to dryness, refluxed for two and one-half hours with 2 per cent. HCl, and then filtered and the filtrate neutralized. Reducing sugars were determined on this filtrate. Hydrolyzable polysaccharide is therefore expressed as reducing sugar.

DETERMINATIONS ON EXPRESSED JUICE.—The entire lot of cane from each harvest was extracted in a three-roller electrically driven "Cuba mill." The extraction is known to be about 50 to 60 per cent. It is obvious that the relative proportion of various solid matter in juice must be different from what it is in the total tissue. This needs to be kept in mind when comparing the various data.

REDUCING SUGARS IN EXPRESSED JUICE.—Reducing sugars were determined by the methylene blue method as adopted by the Association of Hawaiian Sugar Technologists (**2**).

SUCROSE IN EXPRESSED JUICE.—Sucrose was determined polarimetrically

using the Walker inversion method according to the directions given in the reference cited above.

ELECTRICAL CONDUCTIVITY.—The expressed juice was filtered through several layers of cheesecloth to remove mechanical impurities. The juice was then placed in a constant temperature water bath at 25° C. and conductivity determined by a simple wheatstone bridge set up employing slide wires.

pH.—This was determined on the expressed juice by the use of the quinhydrone method using apparatus manufactured by the Leeds Northrup Co.

EXPRESSION OF RESULTS.—The results of the chemical analyses are expressed on the dry weight basis. CHIBNALL'S (**6**) recommendation that data should preferably be expressed on fresh weight cannot be applied here owing to the altered moisture relationships from treatment to treatment and from season to season. Equally unsatisfactory would be the "residual dry weight" basis of MASON and MASKELL (**21**). As they themselves pointed out, this method is sound only when dealing with changes over a short period of time and not at all applicable to cases of rapidly growing plants where the character of the tissue is continuously changing. No one method of expressing results can be entirely satisfactory. It is hoped, however, that the data on dry weight basis in conjunction with that on expressed juice and on the absolute amounts will suffice for a proper understanding of the various physico-chemical relationships.

A word of caution is needed regarding the interpretation of data on absolute amounts. As each harvest represents cane from a different row, even though an adjacent row, so each harvest is subject to those variations which are unavoidable even in the most carefully conducted field experiments.

STALKS OF SECOND AND HIGHER ORDERS.—Owing to the small number of these stalks, the data are not so regular as in the first-order stalks. In general, however, there is good agreement between the various orders. The discussions in the following pages therefore apply equally to these higher orders even though they are not always mentioned by name.

## WATER RELATIONSHIPS

The present study offers some interesting data on the water content of the tissue as affected by differential fertilization and by seasons. As the hydration[5] status appears, from the results obtained in this study, to be intimately related to the chemical composition of the tissue, it is proposed to consider the data at some length.

In tables III, IV, and V (section C) and figure 7 the data are expressed

[5] The term hydration is used here in the sense of MACDOUGAL (**20**) and refers to all the water present in the tissue regardless of how it is held.

as grams of water per gram of dry matter. We see from figure 7 that there is a progressive increase in moisture content as the amount of nitrogen is increased; secondly, that within the same treatment there is considerable seasonal fluctuation that affects all parts of the plant including the dry-leaf section; and, thirdly, that there is a gradual decrease in hydration from the top toward the base of the stalk. The chart further shows that in the dry-leaf section itself there is little evidence of a progressive decrease, especially in the high N series, but such a decrease is conclusively

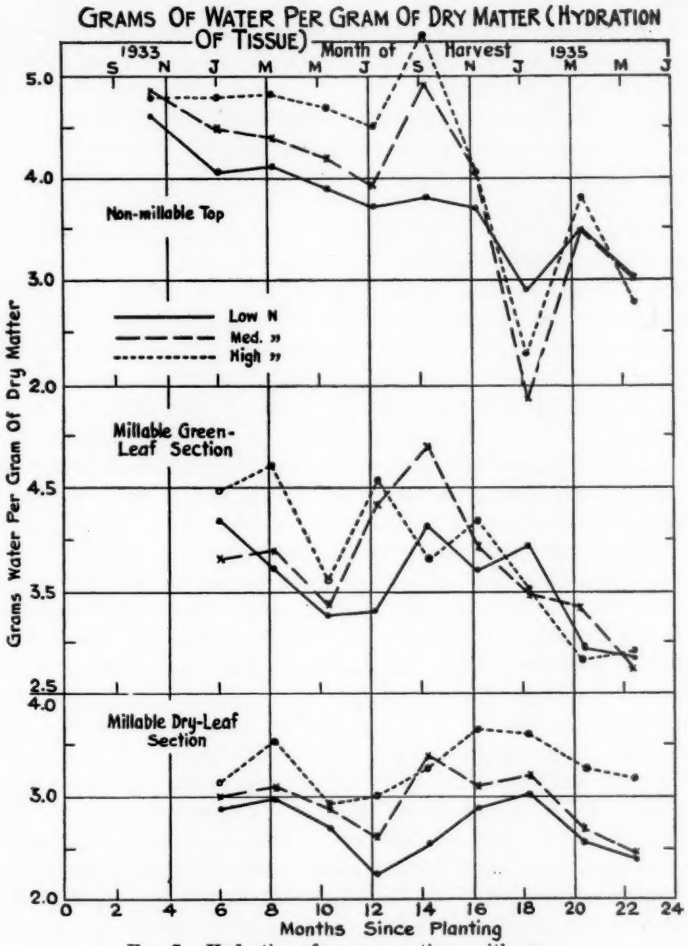



FIG. 7. Hydration of sugar cane tissue with age.

shown in case of the millable green-leaf and the top sections. There is no doubt that the large decrease in hydration in these two sections resulted from cutting off nitrogen after the fourteenth month. The same differences between treatments, seasons, and parts of plants are generally shown by the second-order stalks. The close agreement between the two orders, even in the matter of abrupt fluctuations, leads to the conclusion that such variations cannot be attributed solely to random experimental errors. The differences in water content here brought out are not only of fundamental interest but are also of great practical importance. In the manufacture of sugar the expressed juice has to be evaporated down, and therefore any increase in water content as a result of fertilization means greater production expense.

It is generally recognized that application of nitrogen or other fertilizers increases the water content or succulence of plants; but as to the underlying causes, there appears to be great divergence of opinion. CRIST (**8**) attempted to show that the increased succulence might be related to the hydrophilic colloid content of the sap. Employing the cryoscopic method, he obtained measurements of the bound water content of sap of fertilized plants and found a general relation between the bound water, and by inference the hydrophilic colloid content, and the amount of fertilizer applied. His data were far from consistent, however, and CRIST himself concluded that the bound water explanation was not very satisfactory. A similar colloid explanation was advanced by GARNER (**14**) for tobacco plants. While we know that nitrogen application increases the protein colloids (in the chloroplasts, for instance) and probably the absolute amount of protoplasm in the cells, and while we may assume that the carbohydrate colloids are also increased somewhat, none of these considerations would appear to explain satisfactorily the differences in hydration observed not only in the leafy top but also in the mature dry-leaf section. Furthermore, increase in bound water content may not necessarily mean increase in the content of hydrophilic colloids, for CHRYSLER (**7**) has shown that any increase in the amount of water in a hydrophilic colloid system will automatically increase the absolute amount of bound water in that system.

PEARSALL and EWING (**25**) suggested that the succulence of nitrogen-fed wheat plants might be due to their greater amino acid content and higher pH. They based this conclusion on the work of CRIST on bound water referred to previously, and on the hydration studies of MACDOUGAL with amino acids and biocolloids. Higher pH, according to PEARSALL and EWING, would increase the swelling or hydration of the hydrophilic colloids that were believed to be in greater quantity in the fertilized plants. This does not appear to be the correct explanation, for no consistent differ-

ence in pH between the differently treated plants was found either in CRIST'S or in the present work (see tables III, IV, V, section L). Equally unsatisfactory would appear to be their contention that the amino acids might be a factor of major importance in increased hydration. MACDOUGAL and coworkers (**20**) noted stimulating effects of certain amino acids on a synthetic biocolloid in electrolyte-free solutions only. Apart from the fact that the particular agar-protein mixture used by MACDOUGAL may not be representative of all plant colloids, the effects of electrolytes themselves on hydration cannot be neglected. A consideration of salt, or rather ion effects, became all the more necessary as it is generally found (and as conductivity data (fig. 23) presented here show) that the application of nitrogen increases the absorption of ash constituents.

There are further reasons why amino acids may not be the real explanation. MACDOUGAL showed that the stimulating effects were noted only in great dilution, greater, one would suspect, than that actually obtained in the high N plants of this experiment. Also, in the case of the high N series one should find continuously increasing hydration with age, as the percentage of soluble nitrogen (and by inference of amino acids) was continually increasing. The hydration, however, follows a course independent of that of amino acids. In looking for factors that might explain more satisfactorily not only the differences in water content between treatments but also those between seasons and between parts of plants, it was thought advisable to concentrate first on the dry-leaf section. This part of the plant may be considered relatively fixed. Whereas there may be variations in moisture content of the top and millable green-leaf sections from harvest to harvest owing to the empirical method of separating the two parts, no such source of error is involved in the case of the dry-leaf section, the limits of which are precisely defined. Whereas the top section consists of different kinds of tissue in blade, sheath, and growing tip, and each one probably in a different state of hydration, and whereas even the millable green-leaf section possesses tissue of varying degrees of maturity, the dry-leaf section contains only the mature tissue. Elsewhere (**10**) I have shown that the dry-leaf part is more homogeneous throughout its entire length than are the other two sections of cane here discussed.

Figure 7 shows that the differences due to treatment are the same in the dry-leaf section as in the other two sections. However, these differences are more striking and clear-cut in the former. Confining our attention entirely now to this section, it will be observed that, in general, all the three treatments decline to a low hydration value in the months of May, June, and July, following which there is a definite and general increase in hydration up to the month of January (age twenty months) and then a

second decline. Furthermore, the data show that the hydration in November to January of the second year was slightly greater than at any previous period. As has been stated earlier, no difference in pH or amino acid content can explain the rhythmic variations that appear to be so markedly correlated with seasons. Forthwith, the question arises whether differential absorption of salts may not have some relation to these variations, for such differential uptake is known to take place in different seasons.

A study of the conductivity data shows that we may be on the right trail. The conductivity curves in figure 23 for the dry-leaf sections are in general so similar to the hydration curves, not only in regard to treatment differences but also in regard to seasonal variations, that there appears to be strong indication of a cause and effect relationship. Since conductivity is a measure of ions in solution, it may be postulated that the seasonal variations in hydration are due to the hydration effects of ions. Before proceeding further in support of our postulate, it is necessary to distinguish between what MICHAELIS (**22**) has termed direct or electrostatic effects of ions and the indirect or lyotropic effects of ions. In the former cases the sign of charge and valency are the chief determining factors but in the latter case only the specific chemical nature of the ions becomes manifest. Without going into detailed discussions which can be found in the text referred to, it may be said that in the present study the effects of ions dealt with are probably primarily of electrostatic nature. MICHAELIS has also shown that the lyotropic effect, which might have dehydrating effect on the tissue, becomes operative in high concentrations only, greater than 0.1 N at any rate. The highest figure for conductivity recorded at any harvest for the dry-leaf section is $4.3 \times 10^{-3}$ mhos. Assuming the solution contained KCl only at 25° C., the concentration of the salt would be about 0.03 N. Even in the top section, the salt concentration on the basis of KCl could not have been much higher than 0.1 N except in January of the first and the second year at the age of six and eighteen months respectively. Now in electrostatic effects the factors to be considered are the sign of the charge, valence, and the specific chemical nature of the ions. Tissue colloids will selectively adsorb oppositely charged ions, the degree of adsorption increasing geometrically with valence. Because of this greater adsorption, ions of higher valence will exercise the greater influnce on hydration.

The plant colloids being negatively charged, the cations will be preferentially adsorbed. These adsorbed ions will, through their specific chemical nature, attract a shell of water around the colloid micelle. The anions associated with the cations, but largely unadsorbed by the tissue colloids, will also remain in the field of attraction of the cations and according to their specific nature increase the hydration of the already hydrated micelle.

That the directly unadsorbed anions are not without their influence on hydration is shown by the studies of BRIGGS (**5**) on the electrokinetic potentials of colloid systems. Such anion effects are also postulated by KATZ (**17**) for the increased swelling of cellulose in solutions of various salts. KATZ has further shown that cellulose adsorbs more ions, and hydrates more, the greater the content of salts in solution. This holds true even in mixtures of salts, a condition found in the plant sap. Speaking of such mixtures, MICHAELIS (**22**) states, "where the direct electrostatic effect of ions occurs and the valency rule holds, only a summation of ion mixtures is to be observed; antagonism never occurs." Undoubtedly an estimate of total summation of ions is what we get from the conductivity data. Thus there is strong evidence to indicate that not only the relative hydration of tissue in the three series of treatments, but also the seasonal variations in the dry-leaf sections, may be due to a differential uptake of ions and their hydration effects.

Next we may consider the hydration curves of tissue in the top and the millable green-leaf sections (fig. 7) and compare them with the appropriate conductivity curves. At first glance there appears a lack of similarity in the conductivity and the hydration curves unlike what was found earlier for the dry-leaf sections. The conductivity curves show the same seasonal variations in all parts of the cane. On the other hand, the hydration curve for the top shows a steady declining trend, that for the millable green leaf the same trend, possessing however some features in common with that of the dry-leaf section.[6] On closer observation one notices some parallelism between the conductivity and the hydration data of the top and the millable green-leaf sections. Like the hydration curves, the conductivity curves show progressive even though less pronounced decline with age. Around March and May, at the age of twenty to twenty-two months, the hydration values for the green-leaf section fall to that of the dry-leaf section, as does the conductivity value. One notes, however, that even though the hydration value in the top equals that of the dry-leaf section, the conductivity is still about five times higher.

In attempting to elucidate the differential course of hydration in the three sections, it should be kept in mind that the tissues in the various sections are of different composition, and must therefore behave differently toward electrolytes. In the top the carbohydrate and the protein colloids are probably of somewhat different nature and they undoubtedly exist in different ratio to each other than is the case in the dry-leaf section. Table I

[6] It should be stated that the difficulty in the top section must be due partly to the fact that in the extraction of juice by the "Cuba bill" the blades passed through entirely uncrushed, so that the conductivity data do not include the blade tissue, but the data on hydration do.

shows that there is about five times as much nitrogen in the top as in the dry-leaf section, and of this total nitrogen a much larger proportion is in insoluble form in the top. In the dry-leaf section, therefore, the colloids must essentially be of carbohydrate nature. The millable green-leaf section should stand somewhat in between the two extremes. MACDOUGAL (**20**) has pointed out that the relative proportions in which a carbohydrate and a protein colloid is mixed determine to a large extent the behavior of the mixture toward electrolytes, acids, and bases. We may therefore expect a different course of hydration in the top than in the other sections. Moreover, the greater concentration of salts in the top section and the greater acidity must have profound influence on the surface properties of the tissue colloids.

Also, as AYRES (**3**) has shown, the relative content of the various electrolytes must vary from one section of the plant to another and from one season to another, owing to differential mobility of ions and to seasonal variations in the uptake of salts. A detailed study of AYRES's results indicates that the influence of ions may be dominant even in the top and the green-leaf sections, even though the hydration data may not be so readily correlated with the conductivity curves.

AYRES's data further support the theory that the ions may be the major factor in hydration. He found the total ash concentration to increase from the base of the stalk upward, the highest concentration being obtained in the growing point. Unpublished data on the moisture content of the various parts of the same stalk indicate an exact parallelism.

There is still another indication, although an indirect one, which points to the relation between conductivity and hydration. GORDON (**15**) has recently shown that the concentration of sucrose in the cane juice can be closely determined by the use of a conductometric device. It will be shown later in this paper that there is a marked quantitative relation between hydration and the seasonal concentration of sucrose in juice. In other words, the findings of GORDON are to be explained by the relation between conductivity and hydration postulated above.

It is interesting to recall that KRAUS and KRAYBILL (**18**) considered the possibility of $NO_3$ ions being lyotropically effective in causing greater succulence of tomato plants. Since sugar cane is known from the work of HARTT (**16**) and from other unpublished data to be practically free of any inorganic nitrogen, differences in hydration may not be considered the effect exclusively of $NO_3$ ions. Rather, in view of our data, a summation effect of several or many ions must be regarded as being responsible for the differences observed.[7]

[7] It should be noted that seasonal changes affect the hydration of tissues, not only by altering the concentration of ions but also by purely local effects such as temperature,—

While the foregoing discussion emphasizes the dominant rôle of ions on tissue hydration, it is realized that much more experimentation will be necessary before this point can be clearly established. Should this theory prove essentially correct, however, it will provide a simple and direct explanation for the increased succulence observed when many non-nitrogenous salts are applied to the soil. This will also explain increased water content in shaded or etiolated plants, for it is generally known that shading favors increased salt absorption.

It is beyond the scope of this paper to discuss the different factors involved in the seasonal variations in salt uptake. The subject is dealt with rather exhaustively by LUNDEGÅRDH (**19**) in his recent text.

Before leaving the subject of hydration, a significant parallelism between it and the rate of growth curves (fig. 2) should be pointed out. We shall consider the hydration of the green-leaf section, as this is the section which, more than the top with its varied tissue (or the dry-leaf cane with its old tissue), may be expected better to reflect the seasonal rate of elongation. From a high value around January to March 1934, the hydration in all of the series drops to a minimum value in the beginning of May at the age of ten months; it rises again rather sharply to a maximum in August and September and declines again. Exactly the same course is followed by the elongation curves[8] in figure 2. Rate of growth gradually declines through the early winter months to a minimum value in the first part of May 1934; the curves then turn sharply upward, reaching maximum values in August and September, and then decline again. Even the relative divergence of the growth curves of the three series throughout the seasons and their meeting together again in the late fall of 1934 is similar to that found in figure 2. The top section also follows the same general trend of seasonal variation in hydration as the millable green-leaf section, but with less regularity. The observed parallelisms emphasize the views elaborated by MACDOUGAL (**20**) regarding the significance of hydration in growth.

### ABSOLUTE AMOUNTS OF DRY MATTER

The relative efficiency of the different seasons and the treatments are well brought out in figure 8, which shows the pounds of dry matter in the cane harvested periodically.

In the top section, despite greater fresh weight, the high N series appears

the higher the temperature the greater the swelling of tissue colloids, other things being equal. In other words, temperature must play a dual rôle, first in regulating the absorption of ions, and secondly in increasing the hydration of the entire colloid system, *i.e.*, tissue plus the adsorbed ions. How close the seasonal course of hydration follows that of air temperature will be seen from table I.

[8] As has already been pointed out, elongation is closely related in cane to volume growth.

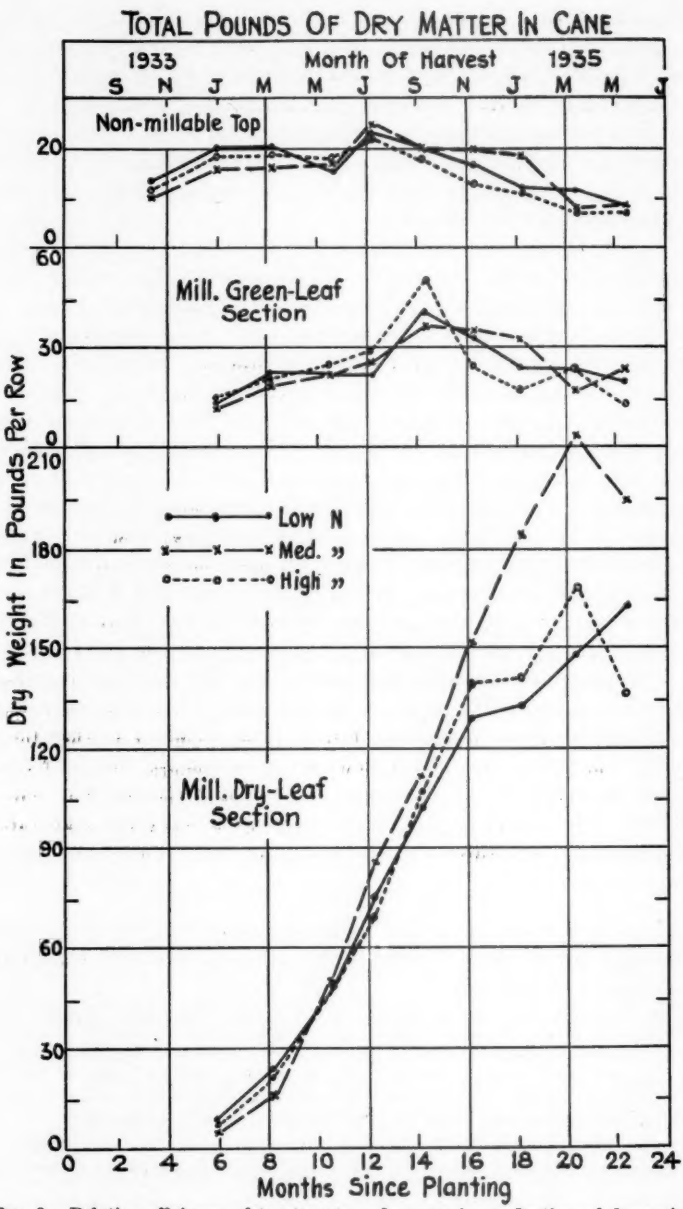



FIG. 8. Relative efficiency of treatments and season in production of dry weight of cane.

to have less pounds of dry matter than the low. After the tenth month the medium N series takes the lead and maintains it for the rest of the season. It would appear that the rate of increase in the absolute amount was the highest around July (age twelve months), which agrees well with the data on the rate of elongation at different periods.

The green-leaf section reflects the seasonal differences even more markedly. The harvest in September (age fourteen months) actually contained a higher amount of dry matter in all of the series than at any period earlier or later. The relatively low concentration of sucrose in the cane of this harvest (fig. 10) does not, therefore, imply less photosynthetic activity. In the dry-leaf section, the superiority of the medium N series over the others is clearly established. From the tenth month on there is an increasing separation of the curves, and after the sixteenth month, the medium N series keeps on steadily increasing while the low and the high N series show large decline in the rate of increase. In the case of the low N series, the decline is no doubt due to the lack of plant food, but in the case of the high it is due to the increasing mortality of cane, induced in all probability by too much fertilization. It should be expected from the data that as sucrose is the principal single constituent of the dry matter, the medium N series will show the highest amount of it, the high and the low being closely alike. Such is actually found to be the case in figure 11.

## Sucrose

The effect of the differential nitrogenous fertilization on the metabolism of sucrose is naturally the most important problem in this study, not only because as commercial cane growers we are primarily interested in it, but also because sucrose forms the principal storage product in cane, constituting 50 per cent. or more of its entire dry weight.

The data on sucrose are presented in figure 9 as concentration in juice, in figure 10 as percentage of the dry matter (*i.e.*, grams of sucrose in each gram of dry matter), and in figure 11 as absolute amounts in the total weight of cane harvested per line. For convenience of discussion, we shall deal first with the differences due to treatment only.

Differences due to treatment.—Figure 9 brings out the influence of nitrogen on the sucrose concentration in juice, and supports the general knowledge of the cane growers that, as the application of nitrogen is increased, the quality of juice becomes poorer and as a result much more fresh cane is needed to make a ton of commercial sugar. The differences in the concentration are marked, not only in the top and the green-leaf section, but also in the dry-leaf part which contains only the mature tissue. However, the results of the later harvests seem to indicate that the differences due to treatment are beginning to disappear. This is not surprising

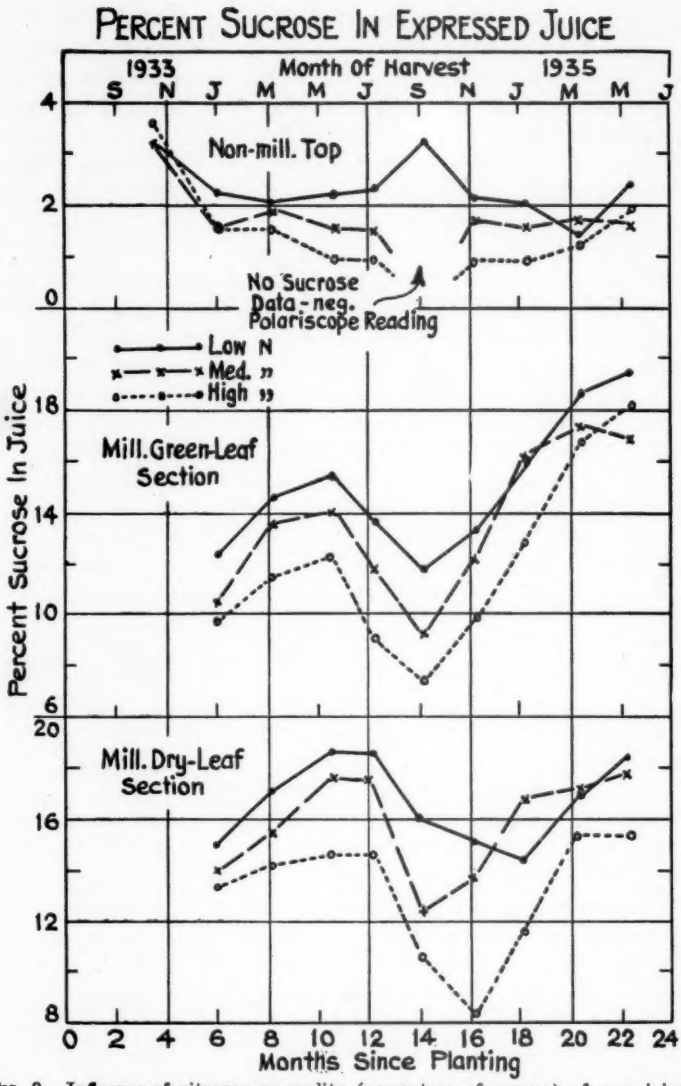

PERCENT SUCROSE IN EXPRESSED JUICE
1933
Month Of Harvest
1935
S N J M M J S N J M M J
Non-mill. Top
No Sucrose Data - neg. Polariscope Reading
Low N
Med. "
High "
Mill. Green-Leaf Section
Mill. Dry-Leaf Section
Percent Sucrose In Juice
Months Since Planting
0 2 4 6 8 10 12 14 16 18 20 22 24

FIG. 9. Influence of nitrogen on quality (percentage of sucrose) of cane juice.

in view of the fact that no nitrogen has been applied since the fourteenth month, thus affording an opportunity for the conditions to be equalized in all of the series.

We have already shown that with increasing nitrogen application there is an increasing amount of water associated with each gram of dry matter in all parts of the plants. It would therefore be reasonable to suppose that the observed differences between the treatments merely reflect the diluting effect of the water. Closer study of the data shows that such is far from being the case. The average hydration of the green-leaf part was 3.56, 3.78, and 3.84 gm. per gram of dry matter for the low, the medium, and the high N series respectively; but the corresponding figures for the percentage concentration of sucrose were 12.96, 11.47, and 9.90. In the dry-leaf part the hydration figures were 2.70, 2.89, and 3.18 and those of sucrose 16.64, 15.83, and 13.07. Clearly, in addition to the differences in hydration, there must have been differences in the content of sucrose in each gram of dry matter in the three series to cause such wide differences in actual concentration. In other words, the higher the nitrogen application the less must have been the proportion of sucrose in the dry matter. Such is actually seen to be the case in figure 10, where the data are expressed on the dry weight basis. There are, then, basic factors connected with nitrogen fertilization that cause the composition of the tissue with respect to sucrose to be different. It will be observed that, although the sucrose content is lower, the content of reducing sugars in the dry matter is invariably higher with higher applications of nitrogen. It will be developed in the next topic that in these differences in the tissue composition we have very likely to deal with an equilibrium between the various carbohydrates of the plant, and that hydration may be the actual regulating factor involved in that equilibrium.

SEASONAL VARIATIONS.—From the practical point of view, seasonal variations in sucrose content are of even greater importance than the differences due to treatment. If by harvesting cane in certain seasons the maximum concentration of sucrose is likely to be obtained, then clearly such seasons should be known and taken advantage of in planning the agricultural policy of a plantation. That the season of harvest has a marked influence on juice quality is generally known. AGEE and MCCLEERY (1) compiled extensive statistics showing that independently of other conditions, quality of juice appeared to be the best in cane harvested in May and June. Specific seasonal influence on cane quality was also postulated by me in an earlier publication (9). However, nothing was known at the time as to how these influences might operate. Some of us were therefore inclined to feel that the observed influences were mere coincidences and had no existence in fact. The present data not only confirm the existence of such specific influences but indicate their mode of operation.

Figure 9 shows that while maintaining their respective levels, the three

series vary considerably from season to season in regard to the concentration of sucrose in juice. Furthermore, the magnitude of the seasonal variations is much greater than the variation due to treatment alone. In the millable green-leaf section, the sucrose content rises to a maximum in May 1934, at the age of ten months, then sharply declines to a minimum value around September 1934, age fourteen months. Following this minimum, the curve of sucrose rises continuously to the age of twenty-two months. There is no doubt that this latter rise is intimately related to the time of last fertilization, which was at the age of fourteen months. In the dry-leaf section the variations are much the same in trend as in the green-leaf section, but the individual treatments differ to a certain extent as to the period of the minimum sucrose concentration. In the top section, the seasonal changes are somewhat different and more irregular than in the millable parts.

To what are the seasonal differences due? We have already seen that there is considerable variation in the amount of water associated with each gram of dry matter in the different seasons and therefore the seasonal variations might be considered at first sight to be brought about by this factor alone.

Again, the data show that hydration changes cannot account entirely for the magnitude of the increase in sucrose concentration up to May and June of 1934 and the sharp decrease around September to November. In addition to the hydration differences, therefore, there must have been actually a difference in the proportion of sucrose per gram of dry matter in the different seasons. This deduction is borne out by figure 10 and tables III, IV, V, section D. There we find that the proportion of sucrose in each gram of the dry matter also increased steadily up to the month of May 1934, and declined rapidly around September and rose again in the following months. In other words, not only do the variations in the concentration of sucrose in the juice in the different seasons bear a marked negative correlation with the changes in the hydration of the tissue, a relationship which is obvious, but also the very composition of the dry matter with regard to sucrose is similarly correlated.[9] Hydration of the tissue, therefore, has much more to do than merely act as the solvent for the sucrose that is present in each gram of dry matter.

[9] The coefficients of total correlation between the seasonal variations in the concentration of sucrose in juice and hydration of tissue are found to be $-0.805$, $-0.707$, $-0.786$ for the green-leaf section and $-0.714$, $-0.880$, $-0.790$ for the dry-leaf sections of the low, the medium, and the high N series respectively. These high correlations may well be expected from the fact that water is the solvent for the sucrose present. However, a comparison of figure 10 (sucrose in dry matter) with figure 7 (hydration) will make it clear that there must also be a high negative correlation between sucrose in dry matter and hydration, although this point will be difficult to confirm statistically in view of the limitations of the data.

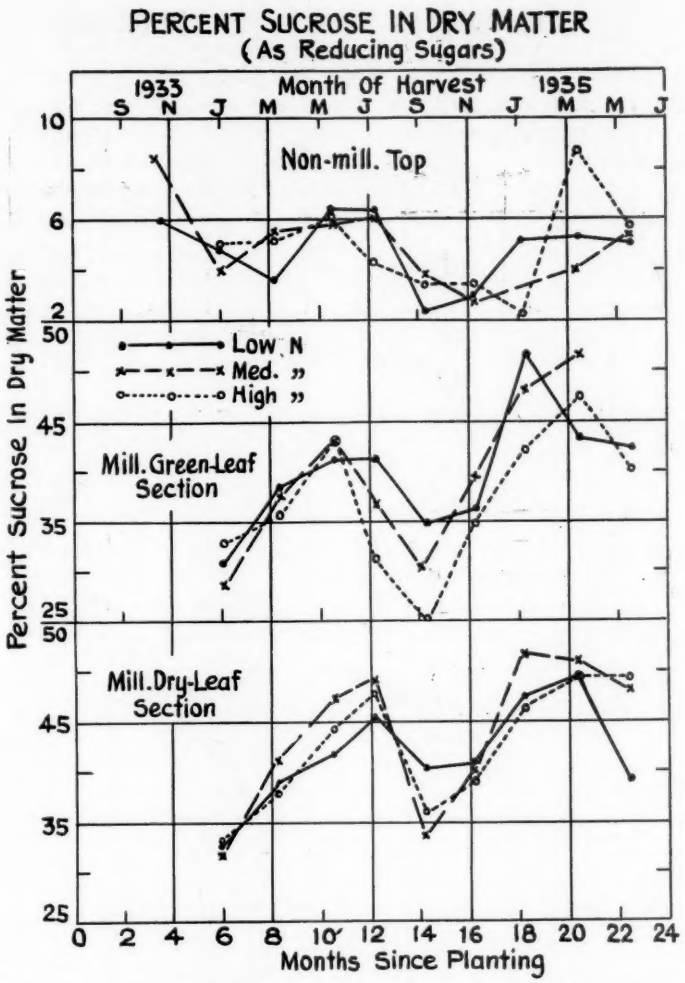



FIG. 10. Influence of nitrogen on the percentage of sucrose in dry matter of sugar cane.

What, then, is the significance of this striking relationship between hydration and the nature of the tissue composition? It it here a case of simple associationship or a cause and effect relationship?

That the hydration status of the tissue is intimately related to the whole growth processes is generally recognized. Considerable evidence has ac-

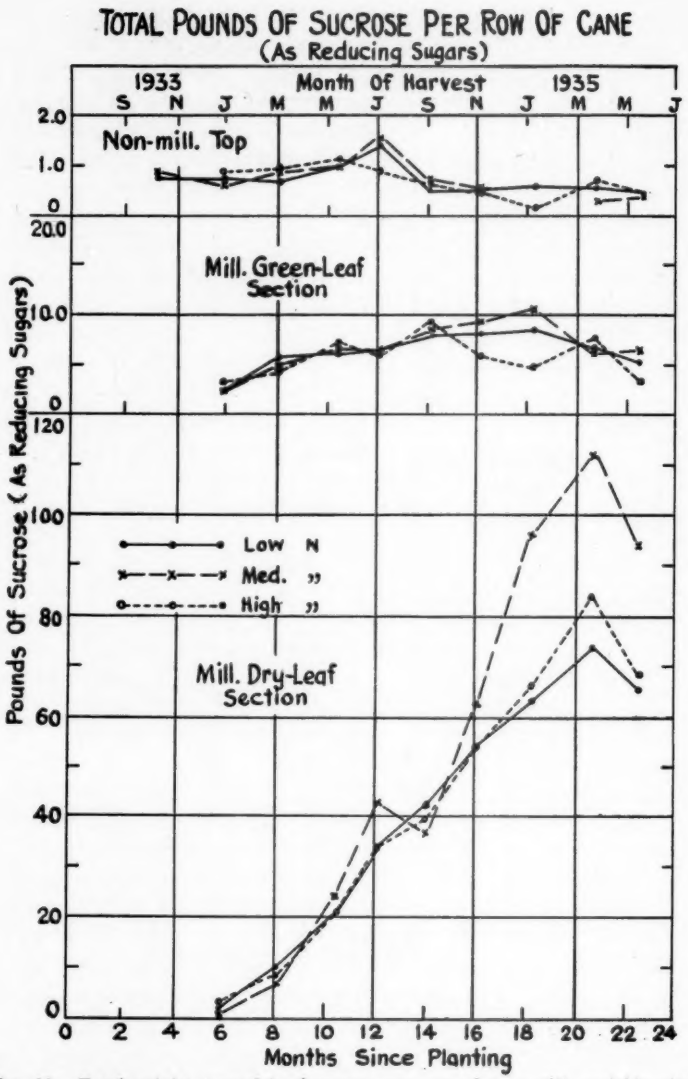


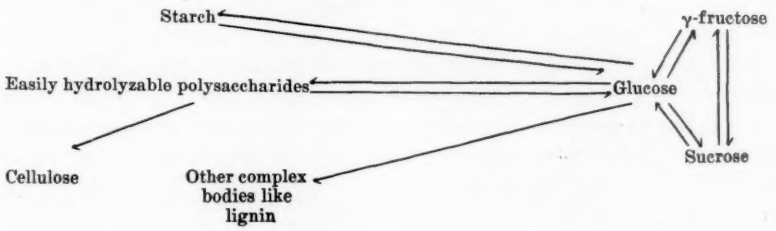

FIG. 11. Total weight (pounds) of sucrose per row of cane with variable nitrogen supply and increasing maturity.

cumulated in recent years showing that the water content of the tissue can exercise profound influence in determining its composition. Thus SCHROEDER and HERMANN (28) found in nasturtium leaves, either unde-

tached or detached from the plant, that desiccation causes an increase in the content of sucrose at the expense of starch and that the phenomenon is independent of light conditions. DE WOLFF (**11**) found the same thing in the potato. This writer further showed that hydration alone, and not temperature, was the causative factor, as the observed increase in sucrose could be brought about by the removal of water from the tissue through purely chemical means. DE WOLFF further noted that increase in the water content caused the proportion of starch in the dry matter to increase at the expense of sucrose. These writers are very probably correct in interpreting the phenomenon as due to the effect of hydration on the complex of the tissue. Even more significant in the present connection is the work of VASSILIEV as cited by RUHLAND and WOLF (**32**). VASSILIEV found "an increase in soluble carbohydrates in wheat plants when water was deficient. On the other hand, with a more readily available water supply, a decrease in soluble carbohydrates was found, despite a continued or increased photosynthetic activity." In our case, it should be remembered that the supply of water to the soil was, throughout the entire experiment, well above the maximum requirements of the plants. The water content of the tissue itself was different, however, and in this last named factor we should then search for an explanation of the rather strange results observed.

SUSSENGUTH (**31**) reports that observations similar to the ones cited have also been made by MOLISCH, RYWOSCH, and others, on different plant species. SUSSENGUTH then proceeds to suggest possible explanations for such a widely occurring phenomenon which appears at first sight to be contrary to the purely chemical conceptions. A discussion of his views, however, will be out of place here.

In the light of these well supported investigations we may believe that there is in plants such an equilibrium as starch $\rightleftharpoons$ sucrose, governed presumably by the enzymes, which in turn are controlled by the hydration status of the tissue. In view of the now accepted constitution of the two substances, however, the equilibrium should probably be written in the form given below rather than the one suggested above:



In other words, sucrose must be considered as the terminal member on the right-hand side of the equilibrium equation and not simply as an intermediate member with a hexose in the terminus. What we are trying to show is that the polysaccharide-to-sucrose transformation probably does not proceed directly but only through the glucose stage;[10] or we may look upon glucose as being at the parting of the ways, one leading to sucrose and the others to glucose and the various polymerization products. Decrease in hydration, it may be postulated, brings about an activation of the enzymes to cause the equilibrium to shift to the sucrose end and increase in hydration to cause a shift in the other directions. (As starch is very inconspicuous in sugar cane, the shift toward the left probably follows the alternative paths shown above.) Indeed there is no logical reason why the equilibrium should stop at the easily hydrolyzable polysaccharide stage and not proceed, under favorable conditions, to carbohydrates of higher complexity, like the cell wall materials.[11]

Extending these observations one step further, hydration may be considered to be an active agency in the regulation of growth and the nature of tissue composition. If the carbohydrates elaborated in photosynthesis are carried to a new cell high in hydration, then they may be expected to move along the path toward the left, forming a larger proportion of the complex carbohydrates and thus further favoring vegetative expansion, whereas under conditions of low hydration the equilibrium will be shifted toward the right, thus promoting an increase of sucrose content at the expense of the other carbohydrates and resulting in slow vegetative growth. Moreover, under conditions of high hydration, reducing sugars and the more easily hydrolyzable polysaccharides may also be expected to increase simultaneously with the still more complex carbohydrates. The present data offer considerable support to the views elaborated above.

Taking first the case of the millable green-leaf cane, where the tissue is more homogeneous than the top and where also the tissue is of very recent origin, we see that the increasing trend in the sucrose content of the dry matter reaching a maximum value in May 1934, age ten months, is exactly paralleled by a decreasing trend in hydration reaching a minimum value in May. The striking drop in the sucrose content in September and the gradual rise thereafter is again perfectly matched by just as striking differences in hydration. We have already noted that the seasonal changes in hydration bear a marked and direct relationship to the rate of vegetative

[10] There is considerable recent evidence that the glucose$\rightleftharpoons$fructose transformation takes place very readily in plants. See MANNE NURMIA, *Nature* **136**: 345; also A. J. VIRTANEN and M. NORDLUND in Biochem. Jour. **28**: 1729. 1934.

[11] However, once the cellulose or similarly complex state is reached, it is doubtful whether the reversal to simpler bodies can take place in the same manner.

growth, and from the various graphs we see that the correlation between hydration and the reducing sugar content on the one hand and that between the latter and the content of sucrose on the other is equally pronounced. Here is then an explanation for the common experience of the sugar planters that under conditions of rapid vegetative growth the sucrose content of the cane is low while that of the reducing sugars is high.

If hydration be the regulating factor in the relative elaboration of sucrose and the other carbohydrates, then we should further expect that as the green-leaf section of today becomes the dry-leaf section in the course of the next few weeks it will further increase in sucrose content owing to the known decrease in hydration, and this increase will be at the expense of the other carbohydrates. Such indeed appears to be the case. The average sucrose content in the dry matter of the green-leaf sections is found to be 39.37, 40.31, and 37.0 for the low, the medium, and the high N series respectively; the corresponding averages for the dry-leaf sections of the harvests two months later being 43.39, 45.61, and 44.18. (It should be noted that owing to the inclusion of the green-leaf data of recent harvests, the difference noted is less than might be expected from vigorously growing cane.) The increase in the proportion of sucrose while passing to the dry-leaf section appears to take place almost entirely at the expense of the reducing sugars for the content of total sugar in dry matter is seen from figure 12 to be practically the same in both the millable green-leaf and the dry-leaf sections. The last observation is significant in that it shows the accumulation of sucrose to be practically complete by the time the joints reach the green-leaf stage. Excellent support is thus given to the view previously put forward (**10**) that the accumulation of sucrose is really very much of a joint-by-joint process and that such accumulation practically ceases when the leaf attached to a joint decays and falls off. However, the few green or partly green leaves that still remain attached to the green-leaf section must be elaborating sugars. We may assume, for simplicity, that such sugars represent the amount that is partly used up in respiration, partly in the formation of fiber in the still soft internodes, and partly stored in the tissues, if we were to consider the slight differences in the total sugar content between the green-leaf and the dry-leaf sections shown in figure 12 to be significant.

The case of the dry-leaf section is rather simple, once the reasons for the variations in the green-leaf section are properly grasped. The dry-leaf section contains, as has been explained before, all of the tissue that was once the green-leaf cane. The seasonal variations in the sucrose content of the dry-leaf tissue must therefore be an accumulative effect, brought about partly by the additive influence of the recently formed green-leaf

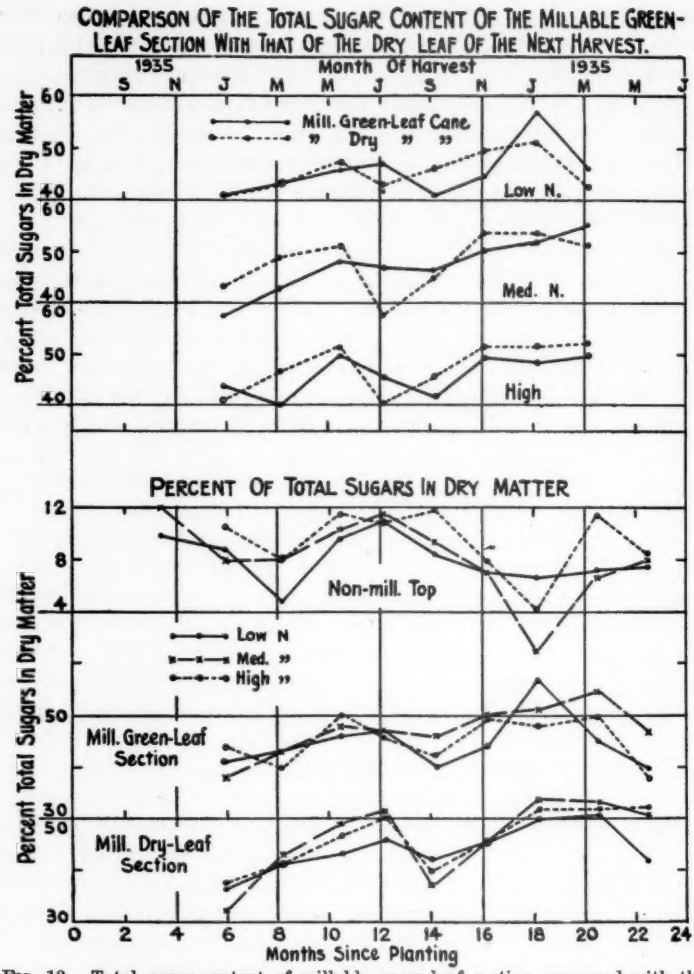

COMPARISON OF THE TOTAL SUGAR CONTENT OF THE MILLABLE GREEN-LEAF SECTION WITH THAT OF THE DRY LEAF OF THE NEXT HARVEST.
1935
Month Of Harvest
1935
S N J M M J S N J M M J
Percent Total Sugars In Dry Matter
Mill. Green-Leaf Cane
" Dry " "
Low N.
Med. N.
High
PERCENT OF TOTAL SUGARS IN DRY MATTER
Non-mill. Top
Low N
Med. "
High "
Mill. Green-Leaf Section
Mill. Dry-Leaf Section
Months Since Planting

FIG. 12. Total sugar content of millable green-leaf section compared with that of the dry-leaf section of the next harvest, dry weight basis.

cane and partly by the interconversions of carbohydrates *in situ* as a result of seasonal changes in the hydration of the tissue. The additive effect is clearly seen in the displacement toward the right of the maximum points of the sucrose curves of the dry-leaf sections as compared with the green-leaf sections (figs. 9, 10). Migration of sucrose *en masse* may probably be ruled out because in the continuously vegetative sugar cane there are no

marked periods of rest following periods of active growth, or *vice versa*, as there is in the deciduous trees of cold climates. Furthermore, it is our common observation that in the case of stalks that flower, the demands of the tassel, as in the case of the variety P.O.J. 2878, appear to be met by a more or less complete depletion of the sugars in a few of the joints immediately below the tassel and not by a moderate withdrawal from all parts of the stalk. The demands of the roots may similarly be met by the joints underground or by one or two of them immediately above the soil surface.

From figure 12 it is also seen that the content of total sugars in the green-leaf section seems to be continuously rising in the later harvests, and correlated with this again is the continual decrease in the hydration of the tissue. No doubt cessation of fertilization at the fourteenth month has to do with the falling trend in hydration and rising trend in the content of total sugars. As the green-leaf cane formed in recent months becomes part of the dry-leaf section, we should expect to find the richness of this portion of the cane reflected in the average tissue composition of the entire dry-leaf section.

In the case of the top section the results are more erratic than in the millable cane, but figure 10 shows that the general trend of the seasonal changes is the same as in the green-leaf section. Undoubtedly the same considerations apply here as in the case of the millable cane. The irregularity of some of the data might conceivably arise from the heterogeneity of the top tissue, consisting as it does of the blades, the sheath, the growing point, etc., or from the fact that the top, being in a state of flux, is harder to define chemically.

Before leaving this subject attention should be drawn to the data on juice in the September 1934 harvest. In the case of the top sections from the first-order stalks of the medium N series and the first- and second-order stalks of the high N series, the polarimetric analysis of juice gave negative readings, suggesting the presence of considerable amounts of levorotatory sugars. Whether their presence implies active respiration or indicates only a certain stage in the carbohydrate metabolism is not clear.

The conception that the hydration of the tissue itself is the regulating factor in the relative elaboration of the various carbohydrates may well explain some of the more recent observations of VASSILIEV (**32**). In studying the effect of weather conditions, he notes that the more favorable the conditions for transpiration the higher the sugar content, and that the fractions of different sugars vary with the weather factors, and further that the age of the leaves has no great importance for the content of carbohydrates. Under conditions of high transpiration, we may logically expect decrease in hydration of the cells, even if it be temporary, and low hydra-

tion will cause the elaborated carbohydrates to move toward the sucrose end. Also, more than the age of the leaves, their hydration will be the determining factor in the relative elaboration of the various carbohydrates.

Although a detailed discussion of it will be out of place here, the point can be brought out that changes in hydration of tissue may indeed be responsible for the increase in sucrose and its winter migration in deciduous trees. It will be noted that in this experiment the seasonal course of tissue hydration was closely paralleled by that of air temperature. The teleological explanations usually offered in the case of deciduous trees certainly do not apply in the case of sugar cane, which was faced neither with winter freezing (the mean maximum temperature at the time of least hydration and greatest sucrose content being 74.3° F.) nor with drought, the water supply being above the maximum requirement. Furthermore, being the natural inhabitant of the tropics, it cannot be said of the cane plant that it ever had any need to develop the so-called protective mechanism of saccharification characteristic of deciduous trees.

In the light of the foregoing discussions it is interesting to observe that the sugar planters in Hawaii have for long entertained the belief that the desiccation of cane growing in the field is a convenient means of increasing the sucrose content. For decades it has been their practice to stop all irrigation about two months before the cane is harvested. Under the semi-arid conditions that mostly prevail in the irrigated plantations, there must be considerable reduction in the moisture content of the tissue which will result not only in an increased concentration of the sucrose already present but also in the amount of sucrose, owing to a shift in the carbohydrate equilibrium toward the sucrose end. Also, under these conditions of deficient moisture (hydration) the newly elaborated carbohydrates must consist very largely of sucrose, thus further increasing the average sucrose content of the whole plant. Significant also is the further observation of the sugar planters that any supply of moisture by rainfall or otherwise to the "dried off" cane results in an increase in the reducing sugar content and decrease in sucrose. Indeed, if hydration of tissue be the controlling factor as here suggested, there is every reason to believe that an increase in the sucrose content may be brought about by a suitable modification of cultural practices.

PURITY OF JUICE AND CANE RATIO.—Another measure of juice quality which is important from the commercial viewpoint may now be considered, namely, the cane ratio or the number of tons of cane required to make a ton of commercial sugar. The cane ratio is a function not only of the concentration of sucrose in juice but also of its purity, which is the ratio of the soluble solids of the non-sucrose type to sucrose. The greater the proportion of these non-sugar impurities, the more difficult it is to recover in the

manufacturing process all the sucrose that there is in the cane. Increasing applications of nitrogen increase these impurities in juice and thereby increase the cane ratio much more than would be expected from the differences in sucrose concentration alone. Consequently a relatively larger percentage of the total sucrose in cane remains unrecovered under conditions of high nitrogenous fertilization.

ABSOLUTE AMOUNTS.—The absolute amount of sucrose (as reducing sugars) in the total cane harvested per row is shown in figure 11.

The efficiency of the respective treatments in the synthesis of sucrose can be determined only from the study of the absolute amount of sucrose and not of the composition of the dry matter. For instance, a certain treatment may show a smaller amount of sucrose per gram of dry matter when compared with another, but the total absolute amount may be greater if there were a considerably greater amount of dry matter produced with the first treatment. Such indeed is the difference between the high and the low N series in the present case, as we see from the data of the dry-leaf section. The absolute amount is generally higher in the high N series despite a much poorer content of sucrose in the dry matter. The medium N treatment is seen to be by far the best. This is not surprising in view of the fact that the amount applied in the medium N series was arrived at after a considerable amount of field trials and is known to be the most productive. Our data support only the previous field experience. The data on the absolute amounts further show that even though the sucrose content of the dry matter was poorest in the September 1934 harvest, there was actually a greater quantity of sugar produced per line around that season than at any time previously. Also, despite a large drop in the content of sucrose, the dry-leaf cane actually gained in absolute amount between July and September, owing to a relatively large increase in the amount of dry matter.

The falling off in the amounts of sucrose in the top and the green sections in the later harvests is due partly to the increasing mortality of cane, as is seen from the data on cane weight in figure 6.

The actual, even if small, decrease in the absolute amount of sucrose in the dry-leaf section of the medium N series at the September 1934 harvest is undoubtedly due to an error in the determination of sucrose in dry matter, for, calculated from the data on expressed juice no such decrease is found; instead a small increase in amount as might logically be expected.

## REDUCING SUGARS[12]

The data on reducing sugars are presented graphically in figure 13 as concentration in expressed juice, in figure 14 as the proportion in the dry

[12] The term reducing sugars denotes all the substances that reduced copper hydroxide. It is believed that the reducing substances are entirely in the form of hexoses, especially

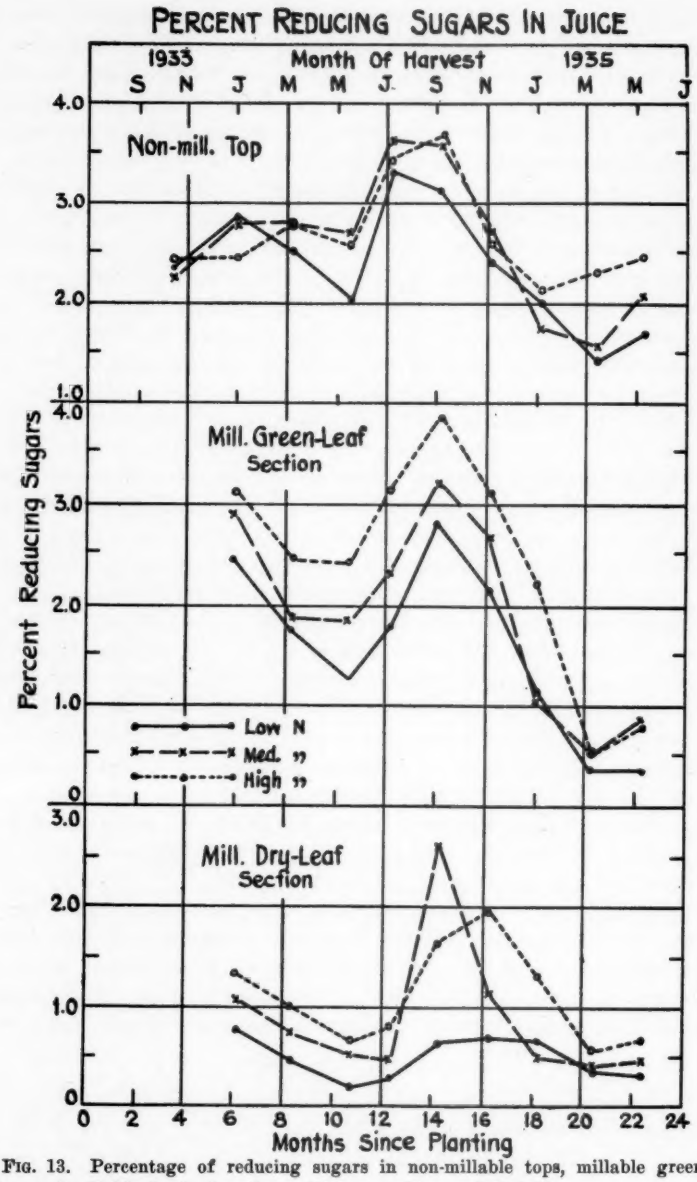



FIG. 13. Percentage of reducing sugars in non-millable tops, millable green-leaf section, and millable dry-leaf section, with low, medium and high N.

matter, and in figure 15 as absolute amounts per row of cane harvested. The actual data are to be found in tables III, IV, V, sections J and E.

DIFFERENCES DUE TO TREATMENT.—Confining our attention to the concentration differences between treatments only, we find in figure 13 that as the amount of nitrogen is increased, so is the concentration of reducing sugars. As there is more water per gram of dry matter with greater applications of nitrogen, we should really expect the results to be the reverse of what is found, provided it were a simple question of dilution. We must therefore conclude that there must have been a much greater proportion of reducing sugars in each gram of dry matter in the high than in the low N treatments. Such is found to be actually the case (fig. 14).

That increased applications of nitrogen increase the reducing sugar content of the tissue has been noted by GARNER and others (**14**) in tobacco. KRAUS and KRAYBILL (**18**), on the other hand, find the reverse situation in the tomato. Conditions specific to the plant dealt with no doubt regulated the relative elaboration of the different constituents.

Under the subject of sucrose, we have outlined our views regarding the origin of such a difference in the composition of the dry matter, and we shall not therefore go into them here. In connection with the enzymic hypothesis there favored, it is interesting to note a recent investigation of DOBY and MAKFALVI (**12**), who find that increasing applications of nitrogen increasingly stimulate the invertase activity in rye and wheat. The stimulating effect of nitrogen may be exercised directly, but in view of our data it is more likely that it is done through a modification first of the hydration status of the plants.

It should be noted that as a result of stopping fertilization, the hydration differences of the tissue are disappearing and simultaneously the differences in the relative proportions of reducing sugars in the dry matter in the three series. Furthermore, the differences between the millable green-leaf on the dry-leaf section is also disappearing in the same manner.

SEASONAL VARIATION.—As in the case of sucrose, seasonal variations are found to be more marked than the variations due to treatment alone. It has already been pointed out that the seasonal variation of the reducing sugar content is exactly the opposite of that of sucrose and similar to that of hydration, of the rate of cane elongation, and, to a less extent, of polysaccharides (2 per cent. HCl soluble) and the "non-analyzed" dry matter. (It will be shown later that this last-named fraction consists mostly of the complex carbohydrates like cellulose and other cell wall materials.) These general similarities have long been recognized by sugar planters and have

glucose and fructose. No other reducing sugars have been reported in cane; and as the cane tissue is known to be very low in starch, there is little reason to suspect the presence of maltose, at least in any appreciable amount.

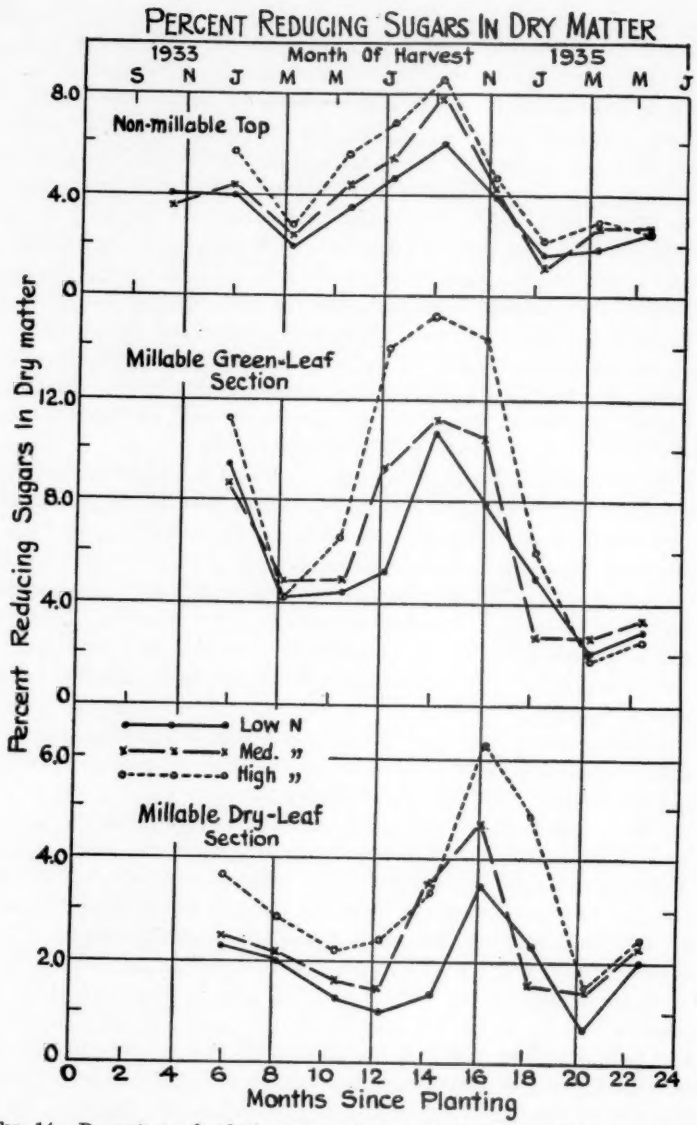

PERCENT REDUCING SUGARS IN DRY MATTER
1933
Month Of Harvest
1935
S N J M M J A N J M M J
Non-millable Top
Millable Green-Leaf Section
Millable Dry-Leaf Section
Low N
Med. "
High "
Percent Reducing Sugars In Dry matter
Months Since Planting
0 2 4 6 8 10 12 14 16 18 20 22 24

FIG. 14. Percentage of reducing sugars in dry matter of non-millable tops, millable green-leaf section, and millable dry-leaf section, with low, medium and high N.

given rise to the wide use of the reducing sugar content of the juice as an index of maturity. The seasonal variations may be attributed to the operation of the same carbohydrate equilibrium as was postulated earlier, and nothing will be gained by a repetition of the arguments.

In the case of reducing sugars, losses due to respiration must not be disregarded, but it will be obvious that such losses cannot modify the trend of seasonal changes. It is interesting to recall that the presence of a large proportion of levorotatory sugars was detected in the tops of the medium and the low N series of the September harvest. If the reducing sugar was fructose, as it is believed to be, then the theory of ONSLOW (**32**) that fructose rather than glucose is used in respiration may find substantiation.

Attention is drawn to a very significant set of data, significant in the sense that they link nitrogen directly with all the manifold differences between treatments and between the seasons we have and are giving to study. We refer to data on the content of total nitrogen in the dry-leaf section. Figure 19 shows the general similarity of the curves of total nitrogen to the corresponding curves of the reducing sugars in juice. Indeed the relationship is found to be significant statistically,[13] particularly in the medium and the high N series. The data, therefore, lend further support to the work of DOBY and MAKFALVI already referred to.

ABSOLUTE AMOUNTS.—Figure 15 shows that with higher applications of nitrogen there is also a greater absolute amount of reducing sugar. This would be expected from the known differences in the yield of cane and the composition of the tissue. As has been said before, more than the composition of the dry matter, the absolute amounts indicate the efficiency of the different treatments or the different seasons. The striking decrease in the amounts following the peak at the fourteenth month should be noted. (It is recalled that fertilization also stopped at the fourteenth month.) There is really no way of estimating how much of this decrease is due to loss by respiration, but, in the dry-leaf sections especially, the loss should not be great. Therefore, the decrease in the absolute amount of reducing sugars in this section after the fourteenth month (decrease in the face of increased cane weight) must be due largely to the interconversion of carbohydrates, as suggested earlier.

### EASILY HYDROLYZABLE POLYSACCHARIDES

Figure 16 (data in tables III, IV, V, section F) shows the percentage of polysaccharides (2 per cent. HCl soluble) in the various parts of the stalk.

[13] The coefficients of correlation between seasonal variations in nitrogen content and the concentration of reducing sugars in juice are found to be + 0.797, + 0.722, + 0.454 for the high, medium, and low N series. The first two coefficients are clearly significant, the last one somewhat doubtful.

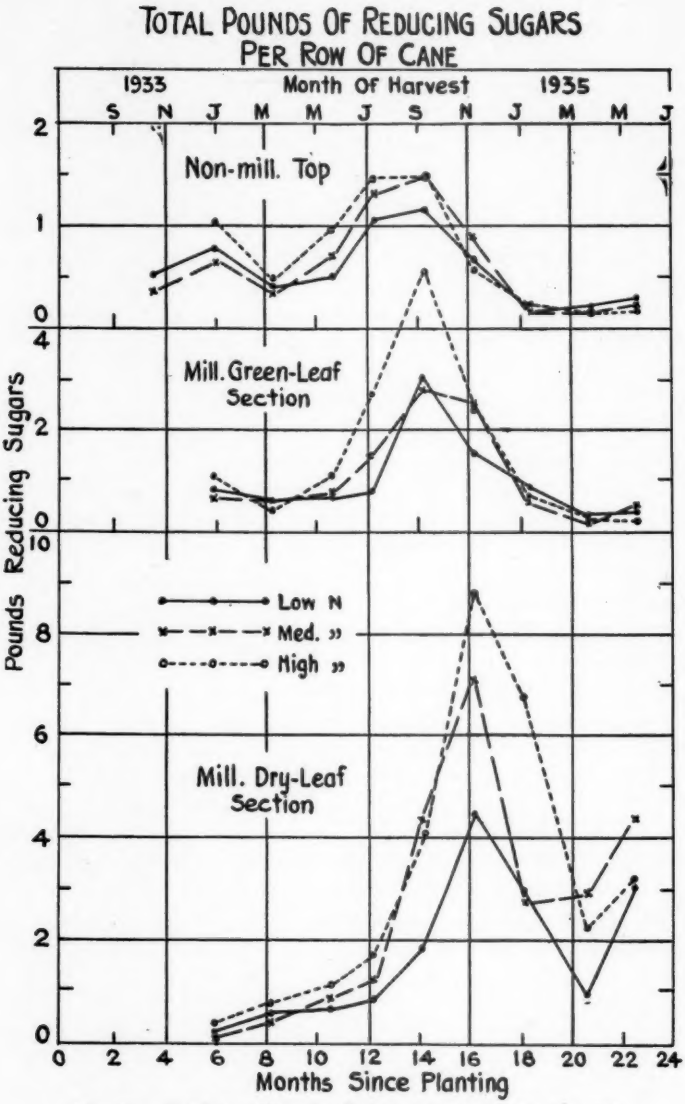



FIG. 15. Total quantity of reducing sugars per row of cane.

There does not appear to be any very consistent difference between the different treatments. There are, however, fairly well defined seasonal variations. In general the trend of these variations is the same as that of the reducing sugars and of the non-analyzed dry matter. Also, as was pointed

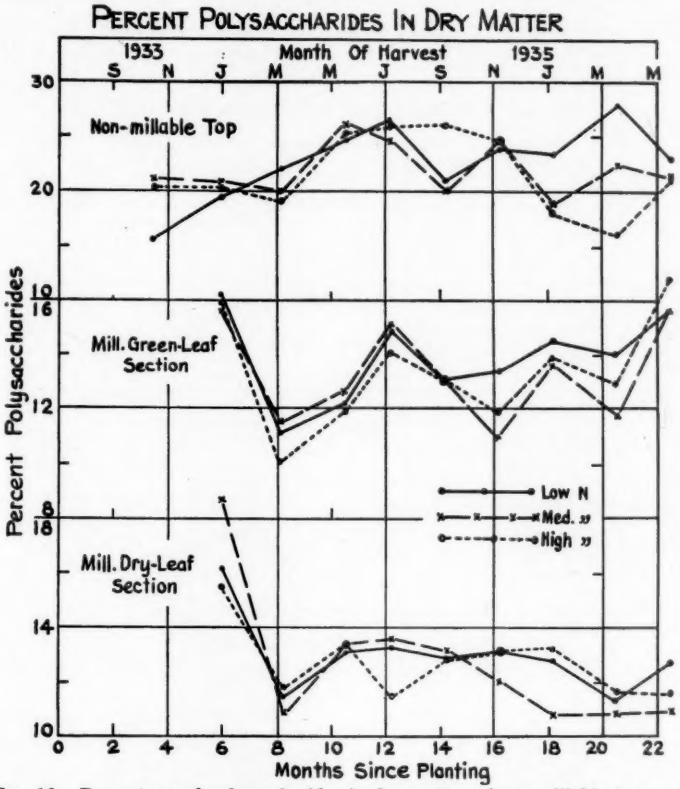



FIG. 16. Percentage of polysaccharides in dry matter of non-millable tops, millable green-leaf section, and millable dry-leaf section, with low, medium, and high N.

out before, the trend parallels the rate of growth curve to some extent. It was suggested earlier that these polysaccharides occupy an intermediate position in the carbohydrate equilibrium of the cane plant, and may therefore be looked upon as the building blocks for cellulose and other complex carbohydrates.

In agreement with this view may be the much greater concentration of these polysaccharides in the actively metabolizing top tissue as compared with the fully developed green-leaf or the dry-leaf tissue.

"NON-ANALYZED" DRY MATTER.—This fraction includes all the dry matter minus the sugars and easily hydrolyzable polysaccharides. In the case of the top tissue, this must include appreciable amounts of ash constituents and nitrogenous substances; but, for the greater part, this must consist of the higher carbohydrates like cellulose and other cell wall materials. In the millable cane, where there is little nitrogen or ash, the fraction must consist almost entirely of the complex carbohydrates. In figure 17 are

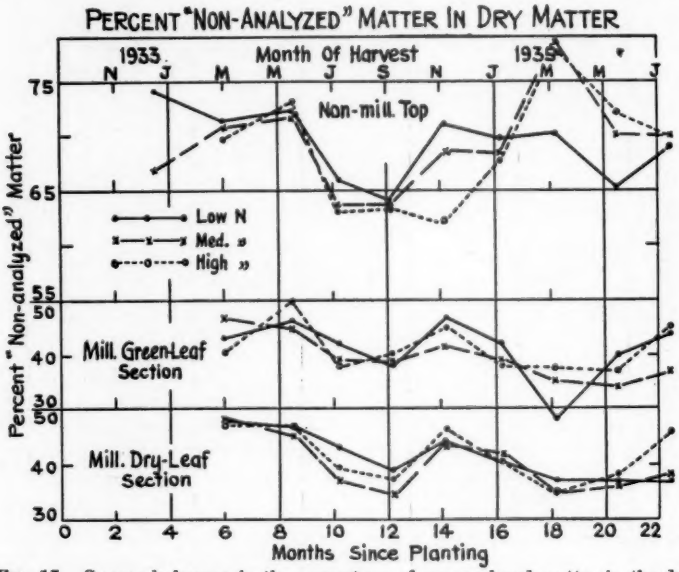



FIG. 17. Seasonal changes in the percentage of non-analyzed matter in the dry substances of sugar cane.

shown the seasonal changes in the proportion of "non-analyzed" matter in each gram of dry matter. There is a general agreement between the seasonal variations here noted and those in the content of reducing sugars and the "easily hydrolyzable polysaccharides." The nature of the carbohydrate equilibrium in sugar cane, sucrose on the end of one path and the other path leading mostly (in the absence of starch) through reducing sugars and the "easily hydrolyzable polysaccharides" to carbohydrates of higher complexity, is again emphasized.

## NITROGENOUS SUBSTANCES

TOTAL NITROGEN.—Figure 18 and tables III, IV, V, section G, show a very definite relationship between the amount of nitrogen applied and the

percentage of nitrogen in the tissue. The differences are equally marked in the top, the millable green-leaf and the dry-leaf sections. The differences show as early as the first harvest at three and one-half months of age, and

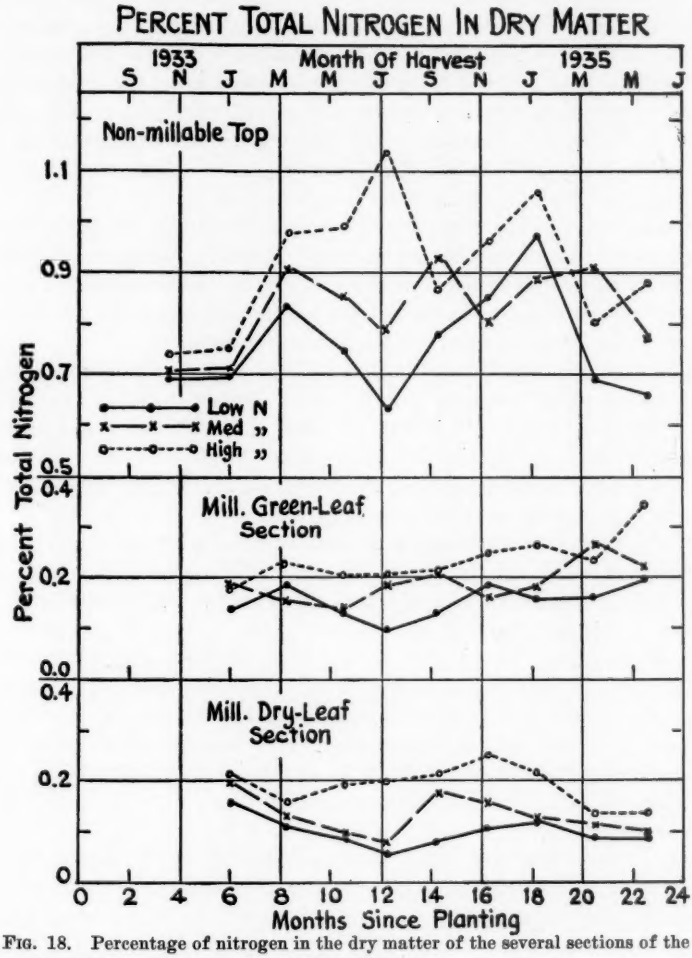



FIG. 18. Percentage of nitrogen in the dry matter of the several sections of the sugar cane plant with low, medium, and high N fertilization.

they tend to become pronounced with the advancing age of the field. However, there are indications in the later harvests as though all the series were approaching each other in composition. This is no doubt the result of stopping fertilization at the fourteenth month.

The difference in the composition of the tissue in the green-leaf and the dry-leaf sections is of interest in that it probably indicates the migration of nitrogen toward the active center of metabolism, *i.e.*, the top. The average of the first eight harvests shows the composition of the green-leaf sections to be 0.154, 0.190, and 0.224 per cent. in total N in the low, the medium, and the high N series respectively. The composition of the dry-leaf section for the same harvests is found to be 0.114, 0.138, and 0.198 per cent. N. The loss in composition therefore amounts to 26, 27, and 12 per cent. respectively, for the three treatments. The relatively small migration in the high N series may be significant in view of the high concentration of N already existing in the top part of the plants of this series.

It is known that in the process of becoming dry-leaf cane, the green-leaf section loses all or most of the chloroplasts contained in the epidermal layers. It may be, then, that the difference between the green-leaf and the dry-leaf sections denotes the chloroplast proteins that have been hydrolyzed and carried to the top to be resynthesized into protein.

It has already been brought out that there is a striking similarity between the trends in reducing sugars and of total N in the dry-leaf tissue. The similarities extend both to the treatment and to the seasonal variations, and undoubtedly signify the close relationship which we have noted before. The significance of the findings of DOBY and MAKFALVI (**12**) in this connection was also pointed out at the time.

Of equally great significance is the similarity of the total N and the conductivity curves in this section of the cane. The conductivity curves, in their turn, have already been shown to be related to the hydration curves, and the hydration curves to the sucrose curves. For the convenience of study, these separate data have been brought together in figure 19.[14] There we must note not only the broad similarities in seasonal trends but also similarities in the sudden changes such as are found in the medium N series, for instance, at the age of fourteen months. One feels as though here in the old tissue the complexities stand partly unraveled, thus affording a rather clear view of the interrelationships through which nitrogen modifies the character of the tissue.

ALCOHOL SOLUBLE NITROGEN.—Data in tables III, IV, V, section H, show essentially the same differences between the treatments as was noted in the matter of total N content. The case of the high N series, however, is much more striking than was the case with total N. There are also other interesting differences between the treatments. In the medium and the low N series, the top is the highest in soluble N, the green-leaf section next, and the

[14] One may not infer from figure 19 that the protein colloids are responsible for hydration, for there is only a very insignificant amount of N in the dry-leaf section and of this low amount only a fraction is insoluble or protein N.

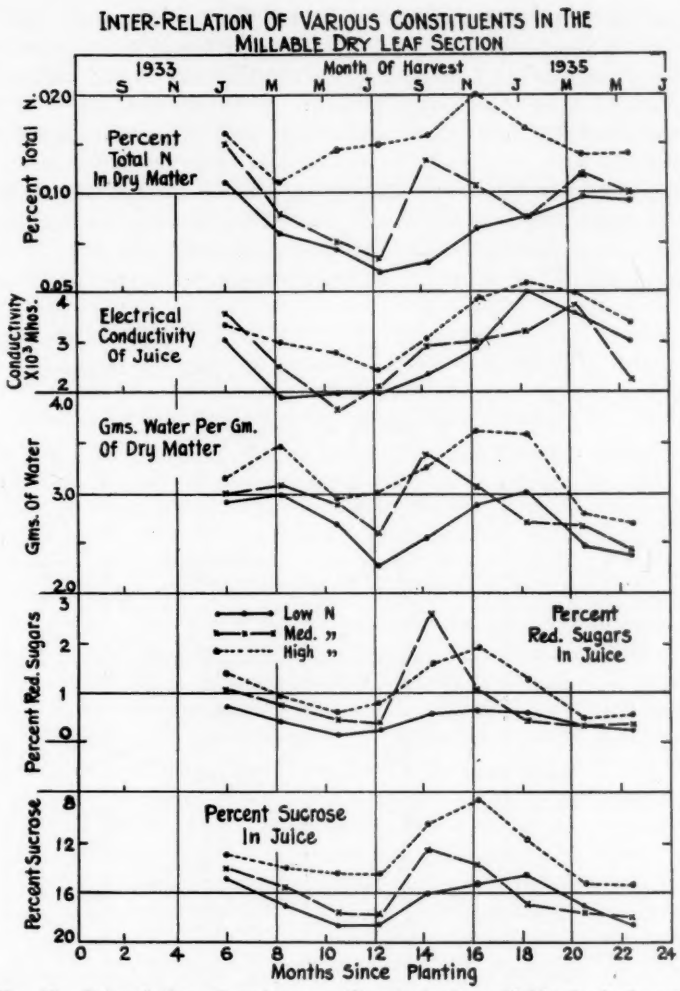



FIG. 19. Interrelation of various constituents in the millable dry-leaf section of sugar cane.

dry-leaf section last. As was postulated in discussing total N, these differences probably imply the migration to and greater concentration in the active top. In the case of the high N series there has apparently been an excess accumulation of soluble N, with the result that the concentration in all parts of the plant has reached the same high level.

The fall in the soluble nitrogen content of the plants from the later harvests may be due to the fact that as there is no external supply of nitrogen there is no renewal of the amount that is being continually used for the formation of protein in the new tissue. It is not clear from the data whether the abrupt fluctuations observed are due to seasonal factors, or to other causes.

RATIO BETWEEN TOTAL AND SOLUBLE NITROGEN.—A point of significance is the generally higher ratio of the total to soluble N in the plants of the low and the medium N as compared with the high N series (**3**). (See figure 20, and tables III, IV, V, section M.) The difference is observed in the top as

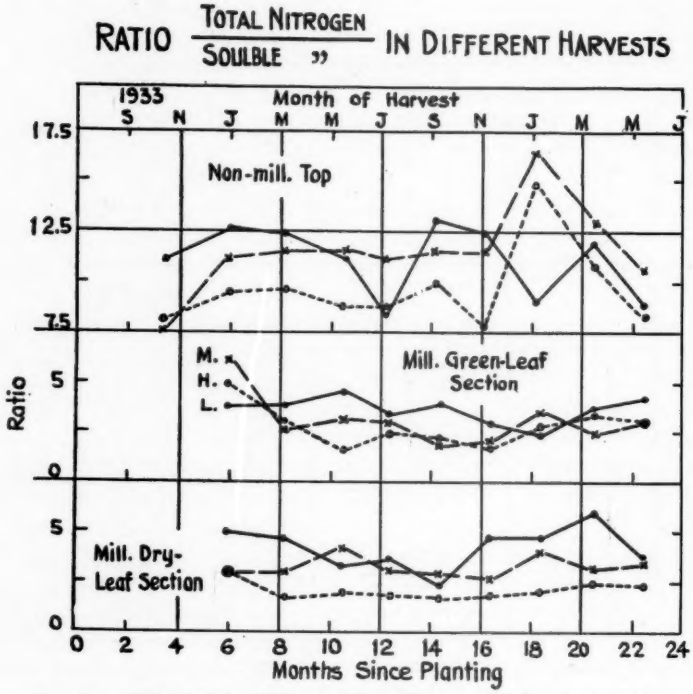



FIG. 20. Ratio of total N/soluble N in sugar cane of different harvests.

well as in the millable cane, and in the first order as well as in the second order of stalks. Such a higher ratio has been noted by MOTHES, as cited by ONSLOW (**24**) and by NIGHTINGALE (**23**) in plants grown in shade as compared with plants grown in full light. Probably light plays only a secon-

dary rôle here as the observed differences in our case could not probably be attributed to differences in light conditions.[15]

There are reasons to believe that the soluble N consists largely if not almost entirely of amino acids. Thus HARTT (**16**) found the amino acid content in the stem of the same variety of cane (H 109) grown in water culture to be almost one-third of that of total nitrogen. In the blades, the same writer found the ratio of total N to amino nitrogen to be about 13. These ratios are rather close to what were actually obtained in the low and the medium N series in this study. The same writer found no inorganic nitrogen or only a trace of it in any part of the cane plant. It should also be expected from the work of ENGEL and others cited by ONSLOW (**24**) that owing to the presence of a large carbohydrate supply in cane there will be little opportunity for amide formation and consequently there may be a maximum concentration of amino acids.

The presence of a relatively larger proportion of soluble N, be it mostly amino acid or not, may or may not signify inability of the plant to form protein N. If inability, it could not be due to the lack of carbohydrates, for the high N plants were about as rich in them as were the plants in the other treatments. Furthermore, it should be remembered that the high N plants were actually growing at a faster rate and presumably experiencing no difficulty in synthesizing proteins. In view of the already noted effect of nitrogen in promoting invertase activity, it may not be unreasonable to suggest that the large concentration of soluble nitrogen encountered in the high N series may be due to overstimulation of the proteolytic enzymes. Whether the influence of nitrogen would be exerted directly or through a modification of the hydration status of the tissue is a matter for speculation.

RATE OF NITROGEN UPTAKE.—Tables III, IV, V, section G, and figure 21 show the data on absolute amounts of N. From these data has been calculated the amount of nitrogen recovered in all parts of the plants of both the first- and second-order stalks in a single line of cane at each harvest. For convenience of discussion, this amount is shown in pounds per acre in figure 22. This figure shows that the actual uptake is about the same for all the series up to about eight months, but from then on the differential effect of treatments begins to be manifest. The high N series, as might be expected, continued to take up nitrogen at an even rate as long as it was applied, *i.e.*, up to the sixteenth month. In the medium, and in the low N series also, greater and greater amounts were recovered in the succeeding crops until about the eighteenth month, when there was an actual decrease in amount.

[15] It is true that with heavy fertilization the foliage is much denser and less light penetrates into the cane rows, but the tops are still able to reach out into the open. Even though there be mutual shading, the differences between treatments would not probably be so great as is shown in the ratios being discussed.

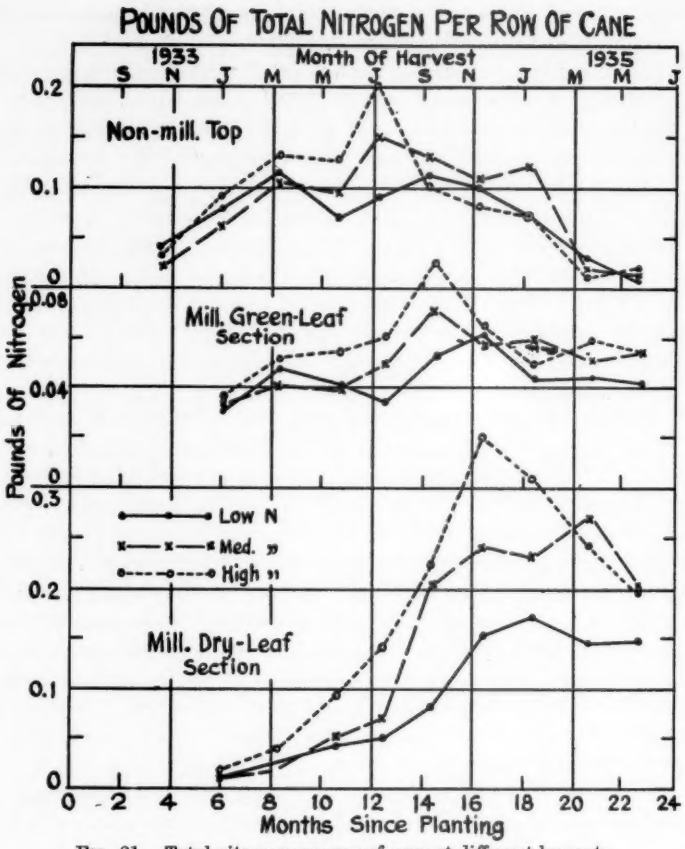



FIG. 21. Total nitrogen per row of cane at different harvests.

This decrease is accounted for by the dead and dying canes which were not harvested or analyzed.

The total amounts of N recovered at each harvest have now been expressed as a percentage of the amount of N actually applied up to the time of harvest (fig. 22 *a*). The result is instructive. In the low N series, there was considerably over 100 per cent. uptake in the earlier months and the uptake is still 100 per cent. in the harvests up to eighteen months of age. This unexpectedly high uptake no doubt explains why the low N plants have grown and yielded so much better than we had thought possible.

As the application of nitrogen increases the relative efficiency of utilization decreases. Thus the average uptake is about 75 per cent. in the

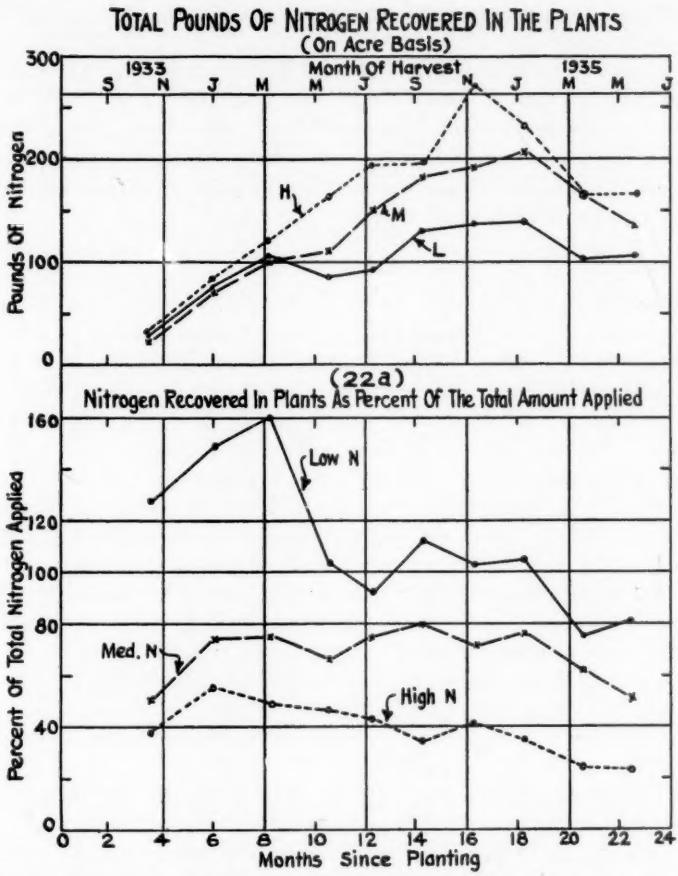



FIG. 22–22a. Total nitrogen per acre recovered in the plants. 22a. Total nitrogen recovered expressed as a percentage of the nitrogen applied.

medium N series and only about 42 per cent. in the high N series. What happens to the rest of the N is not known with certainty.[16] A small fraction is no doubt lost in the dead leaves and another small fraction contained in the higher orders left out of account here, but the addition of these two fractions will make little change in the results. Noteworthy also is the re-

[16] There are reasons to believe that some nitrogen may be lost in the drainage water, such loss probably being greater the higher the application of soluble nitrogenous fertilizers.

markably uniform rate of uptake from season to season in the medium and the high N series.

A point of considerable practical importance may be raised here. If by increasing N we are decreasing the efficient utilization of it, should not the move be toward greater and greater efficiency and not toward still higher applications with attendant waste? The most efficient application of N under the conditions of this experiment is seen to be lower than 266 pounds, probably in the neighborhood of 200 pounds.

### Electrical conductivity

The conductivity of juice (fig. 23, tables III, IV, V, section K) shows more pronounced treatment differences in the dry-leaf than in the other two sections. This is no doubt to be explained by the differential migration of the different ions toward the top. Some striking differences, however, are observed even in the top and the green-leaf section in the earlier stages of the crop. The average conductivity in the top is about $15 \times 10^{-3}$ mhos, in the green leaf about $5 \times 10^{-3}$, and in the dry leaf only about $3 \times 10^{-3}$. Assuming a linear relationship between concentration of ions and conductivity, an assumption that is not strictly correct, there may be found five times as much electrolytes in the top as in the dry-leaf part of the cane.

The seasonal variations are striking and undoubtedly signify specific seasonal influences on salt absorption. The uptake is the greatest in January and least around July and August. The great increase in January 1934 is of particular interest in that it coincides with the period when the cane reached the greatest leaf development and started on the "grand period" of growth. It is also the period when the maximum root development is believed to have taken place.

The parallelism of the conductivity curves in the dry-leaf section to the corresponding curves of total N and of hydration has already been noted. There appears to be an excellent quantitative relation between treatment differences on conductivity and on hydration, as has already been pointed out in discussing the last-named topic. Figure 19 shows a good inverse relationship between conductivity and the percentage of sucrose in juice. This would be expected if, as we have suggested, conductivity influences the hydration of the tissue. The relationships here brought out are so suggestive that it is intended to devote special attention to them in future studies.

### pH

There appears to be no difference in pH due to differential fertilization (tables III, IV, V, section L). However, there are significant differences between parts of plants and between seasons. In general the pH is highest

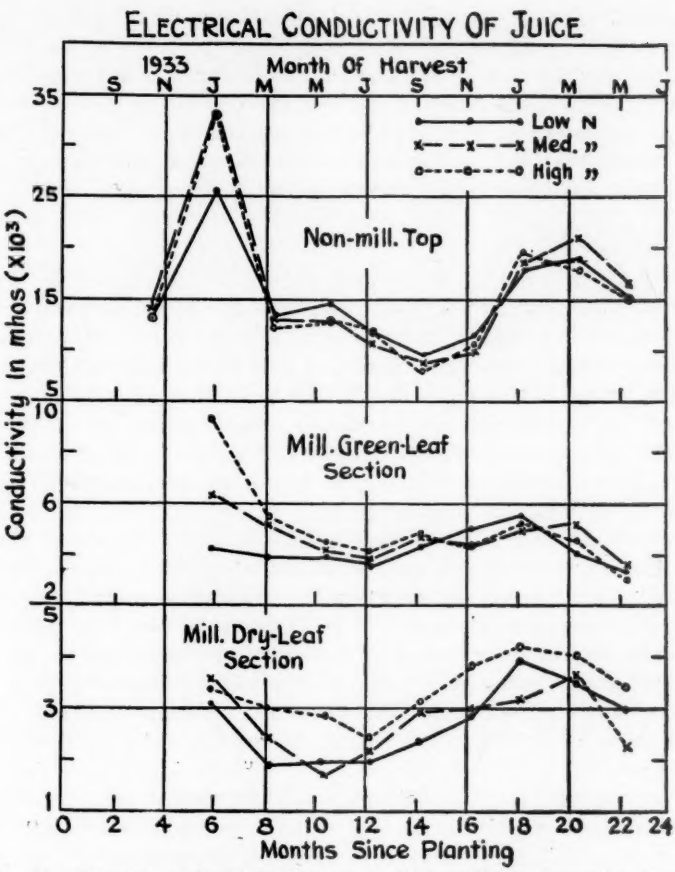



FIG. 23. Electrical conductivity of sugar cane juice at different harvesting dates.

in the dry-leaf section and least in the top, signifying the probable relationship of higher acidity to greater metabolic activity. In accord with this viewpoint would be the gradual increase in pH of the top tissue after the sixteenth month, with coincident decrease in the metabolic activity. In the later stages of growth all parts of the plant appear to be getting alike physiologically, as is shown not only by the data on pH but also those on hydration, conductivity, etc.

The slight but regular trend toward decreasing pH (*i.e.*, increasing acidity) shown in the green-leaf and the dry-leaf sections is not entirely understandable. Are the differences significant, and if significant, what do

they mean? It may be noted that a similar decrease in pH with advancing age was found by GARNER and others (13) on several plant species. We do not know whether there is any accumulation of organic acids in the fully developed cane tissue with age. Should the increase in acidity be found real on further experimentation, it may provide an explanation for the eventual breakdown of the tissue in very old cane, for pH difference might bring about a disturbance in the enzymic equilibrium in the plant.

### REMARKS

Little has been said, so far, regarding the direct influence of the climatic conditions, temperature, sunlight, etc., on the activity of the plant and the composition of the tissue. It must be obvious, however, that such factors as temperature and light play important rôles both directly and indirectly. Directly, by regulating the photosynthetic activity and indirectly by regulating the salt absorption and the various other physico-chemical processes in the plant. The marked linear relation between air temperature and cane elongation has already been noted, as well as the relation of the rate of growth to the composition of the tissue.

Another intimate relationship should be pointed out, namely, that between the effects of nitrogen application and the generally known effects of shading. In both cases the hydration, the uptake of salts, and the percentage of reducing sugars are increased while that of sucrose is decreased. In both cases the leaf characters and the stand of cane are similarly modified. Increased tiller production resulting in increased plant competition may actually reduce somewhat the amount of light received by each plant in the high N plot, but it is not considered likely that this difference alone would cause such far-reaching modifications in the composition of the tissue. It is much more probable that light plays only an indirect part, and that the key to the whole question is the increased absorption of certain salts. The problem unquestionably deserves further experimentation.

## Summary of results

This paper embodies the results of a preliminary study of the effect of nitrogen fertilization on the yield and composition of sugar cane, variety H 109, grown outdoors at the Experiment Station in Honolulu. Three distinct levels of fertilization were employed, a low, a medium, and a high, the total amounts of N per acre in the three series being 133, 266, and 645 pounds respectively. It was found that:

1. Increasing applications of nitrogen increase the size of the leaf, the rate of leaf and joint formation, and the rate of elongation. The stimulating effect on elongation is pronounced only with moderate increases, the

high N series being only slightly better than the medium. Increasing amounts of nitrogen produce increasingly greater lodging in cane.

2. Nitrogen favors tillering and increases the yield of cane. In the high N series the yield was depressed considerably in the latter months, owing to high mortality of cane, leaving the first place to medium N.

3. Nitrogen promotes succulence in cane, *i.e.*, there is more water per gram of dry matter. The hydration of the tissue varies not only with the treatment but also with the season, and to a much greater extent. It is suggested that the treatment as well as the seasonal variations are due, primarily, to the differential absorption of inorganic salts as a result of differential fertilization. Hydration of the tissue is also found to be markedly correlated with the rate of cane growth and with the contents of sucrose and reducing sugars in the tissue.

4. Increasing applications of nitrogen decrease the concentration of sucrose in the expressed juice and less markedly the content of it in the dry matter. Seasonal variations are seen to be of far greater magnitude than the variations due to treatment. Both the seasonal and the treatment variations in the sucrose content are found to be closely associated with the hydration changes. It is suggested that more than merely affecting the concentration of sucrose in juice, the hydration status actually regulates the relative contents of the various carbohydrates in the dry matter, probably through its influence on the enzyme complex in the cane plant. An equilibrium system is postulated to accord with this view. Elaboration of total sugars appears to be practically complete by the time the joints reach the millable green-leaf status. However, a further increase in sucrose is observed to take place later, apparently through the interconversion *in situ* of the reducing sugars. The content of sucrose in the dry matter is inversely related not only to hydration but also to the rate of vegetative growth, and to the content of reducing sugars.

5. Increasing applications of nitrogen increase the content of reducing sugars in the dry matter, also their concentration in the juice. Here, also, seasonal variations are found to be of greater magnitude than those due to treatment alone. It has already been pointed out that the hydration status of the tissue is presumed to be responsible for the differences observed. Close parallelism is found between the content of reducing sugars and the rate of cane growth. There is also a pronounced negative correlation between the content of reducing sugars in the dry matter and that of sucrose. In the dry-leaf section especially, there is observed a marked positive relation between the reducing sugar content and that of total N in the dry matter.

6. The higher the application of nitrogen the greater the percentage of

total and alcohol-soluble N in the tissue. In the high N series there appears to be an excessive accumulation of soluble N. The ratio of total N to alcohol-soluble N is consistently lower in all parts of the plant of the high N series than in the corresponding parts in the other two series. Of the total amount of N applied to the soil during the experiment, 100 per cent. or more was recovered in the plants in the low N series, about 75 per cent. in the medium, and only about 40 per cent. in the high series. The rate of uptake was nearly constant up to the age of sixteen months, especially in the high N series. A close relation between the total N content of tissue in the dry-leaf section and the other constituents was found.

7. Increasing applications of nitrogen produced no clear-cut differences either in the polysaccharide (2 per cent. HCl soluble) content of the tissue or in the pH of expressed juice. Seasonal variations, however, were pronounced.

8. Increasing applications of nitrogen increase the electrical conductivity of expressed juice, especially in the dry-leaf section. The striking relationship between conductivity, hydration, and the concentration of the sugars has already been discussed.

9. Medium N series generally produced a much greater amount of dry matter and sucrose than either the low or the high, which were closely alike. The results thus support the prevalent practice of nitrogenous fertilization in the irrigated plantations of the territory.

10. Although not quite clear in the details, yet the broad outlines of a rather simple picture can be discerned in the results presented here. In the case of the cane plant, application of nitrogen would appear first to increase the absorption of the ash constituents, resulting in a modification of the hydration capacity of the tissue colloids. Alteration in the hydration status, in addition to its direct effect on the concentration of the soluble substances of the cell sap, would appear also to control the relative elaboration of the various carbohydrates in the tissue, presumably through its influence on the enzymic complex of the plant. From this point of view, the marked effect of the seasonal differences in light and temperature on the cane plant may be considered to be exercised both directly by a modification of the photosynthetic rate and indirectly by the regulation of the uptake of salts and the physico-chemical processes in the plant.

Thanks are due Mr. A. H. Cornelison of the Experiment Station staff, who carried on the work during the absence of the writer and without whose painstaking efforts some of the data could not have been gathered.

To my advisers, Drs. R. A. Gortner and C. H. Bailey, I am deeply indebted for many helpful suggestions in the interpretation of the data, and

to Mr. H. P. AGEE, the Director of the Experiment Station, for permission to initiate the research and present the results in this form.

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# SUSCEPTIBILITY OF SEVENTY SPECIES OF FLOWERING PLANTS TO X-RADIATION

EDNA L. JOHNSON

(WITH FOUR FIGURES)

## Introduction

Ten years of study of the effects of x-rays on different species of flowering plants has resulted in a grouping according to ray susceptibility which should be of value, since many investigators are engaged in studying the various ways in which radiation influences living organisms. When sufficient data concerning the morphological and physiological action of x-rays have been collected, it should be possible to give reasons for the ray resistance of some families as compared with the ray susceptibility of others. This paper deals particularly with the morphological effects produced on the above-ground parts of seventy different species distributed in thirty-five families; another paper (**11**) will discuss the action of the rays on underground stems or roots of twenty-two species. The susceptibility of species of *Helianthus, Solanum, Lycopersicum, Vicia, Atriplex, Linum, Nemophila,* and *Zinnia* have been treated elsewhere (**5, 6, 7, 8, 9, 10, 12**).

## Methods

Seedlings were irradiated when the cotyledons were expanded and the growing tip visible. Pots or flats containing irradiated plants and controls were kept side by side on the greenhouse benches, so that all plants were exposed to the same environmental conditions except for the treatment. If the experimental plants were transplanted, the controls were also. Considerable preliminary work was carried on which is not here recorded. Some species were tested which failed to grow normally under greenhouse conditions or which gave such erratic results that the experiment seemed to be of little value. In many cases only one study was made of the species in question, but, with some in which results appeared to be contradictory, experiments were repeated many times.

Data on vegetative growth and blossoming were taken when most of the control plants had completed their growth. The facts set down for each species depended somewhat on the general form of the plant. In all cases the following were recorded: number of plants reaching maturity, age when irradiated, x-ray dose, and total height at maturity. The age in days refers to the number of days from the date of planting. The total height was recorded in all cases as the distance from the soil surface to highest point of the plant. In some cases this meant to the tip of the inflorescence, or, if the plants were still vegetative, to the tip of the highest leaf. In many cases the

age in days when the first blossoms appeared is given. Blank spaces in the columns of the table, where blossoming is considered, indicate a lack of first records of blossoming rather than an absence of flowering. If the plants were branched, records were taken which best indicated how branching was influenced by the radiation, such as percentage bearing laterals, average number of branches, and average length of branches.

In recording the x-ray doses, the r-units are stated for the later ones studied, while the set-up of the machine is given for those treated before a dosimeter was available. Unfiltered rays were used in all cases.

## Observations

The families with the species studied may be grouped into three divisions: (1) species apparently unaffected by treatment; (2) species slightly affected, perhaps for only a short time after irradiation; and (3) species noticeably affected by the rays. Those in the first group so nearly resembled the controls in their growth that figures are not given except in a few cases. Species placed in the second and third groups are treated in greater detail.

### Group I. Species apparently unaffected by x-radiation

The species listed in this group were treated in the seedling stage when the first foliage leaves were beginning to appear. From one dozen to two dozen plants of each species were given what may be considered a medium dose, 80–90 K.V. 5 ma., distance from the target to the growing point 30 cm., for 22 minutes. The plants were watched during their growth for indications of change due to x-ray treatment. Dates of blossoming of both groups of plants were recorded and at maturity the average height and average number of branches were determined. In this group, the experimental plants so nearly resembled the controls that the complete data are not recorded. The species apparently unaffected by moderate exposure to x-radiation were:

Chenopodiaceae
*Blitum capitatum*
Amaranthaceae
*Amaranthus tricolor*
Aizoaceae
*Mesembryanthemum crystallinum*
Ranunculaceae
*Nigella damascena*
Papaveraceae
*Eschscholtzia*
Capparidaceae
*Cleome pungens*
Crassulaceae
*Sedum coeruleum*
Tropaeolaceae
*Tropaeolum majus*
Primulaceae
*Anagallis grandiflora*
Boraginaceae
*Cynoglossum amabile*

Scrophulariaceae
*Torenia fournieri*
Dipsaceae
*Scabiosa caucasica*
Carduaceae
*Aster*
*Emilia flammea*
*Calendula officinalis*

The controls and irradiated specimens listed showed little or no difference in average height of plants at maturity. In most cases the dates of blossoming were the same in both groups. If any of the usual leaf irregularities which accompany the application of x-rays were evident during the early stages, they disappeared long before the plants were mature.

Two of the species listed above need special mention, *Mesembryanthemum crystallinum*, the ice plant, and *Sedum coeruleum*, the stonecrop. A preliminary experiment with irradiated seedlings of the ice plant indicated that irradiation caused no visible change in this species. Later 57-day-old seedlings were treated with 2500 r-units. There were thirty-three controls and twenty-one experimental seedlings which lived to maturity. The irradiated plants showed a gain not only in height, but in average number of branches per plant, and average dry weight per plant. A third study of this species was made, since increase in growth due to radiation is too unusual to record without numerous tests. Of the 69-day-old seedlings given 2500 r-units, seventeen controls and fourteen treated plants lived to maturity. Careful measurements of this group showed in the experimental plants reduction in average height, in the number of plants bearing branches, and in the number of branches produced. The treated plants were later in blossoming, and their green weight at the close of the experiment showed 20 per cent. reduction. Thus, while irradiation produced no visible damaging effect upon this fleshy, thick leaved plant, it is evident that there was no increased growth due to irradiation.

A preliminary test of the stonecrop indicated that irradiation of the seedlings with a medium dose did not visibly affect the development or the blossoming. A second test involving 234 44-day-old seedlings resulted in a slight decrease in the average height of the treated plants and a considerable reduction in the average green weight per plant. The time of flowering was not influenced by the treatment.

These two species, ice plant and stonecrop, well illustrate that although there seem to be in some species no visible effects produced by the rays, there is usually a reduction in amount of plant tissue produced by the treated specimens, as may be demonstrated by a comparison of green weights.

### Group II. Species slightly affected by x-radiation

Species listed in group II are those which in their earlier stages gave evidence of being ray-susceptible, but as the plants matured they showed

## TABLE I

GROUP II. SPECIES SLIGHTLY AFFECTED

| FAMILY AND SCIENTIFIC NAME | AGE WHEN IRRAD. | SET-UP FOR DOSE OR DOSE IN R-UNITS | NO. OF PLANTS | |
|---|---|---|---|---|
| | | | CONTROL | IRRAD. |
| | *days* | | | |
| Chenopodiaceae | | | | |
| *Chenopodium album** | ..... | 84 K.V. 10 ma. 30 cm. 10 min. | 33 | 35 |
| *Spinacia oleracea** | 11 | 91 K.V. 5 ma. 30 cm. 20 min. | 20 | 24 |
| Amaranthaceae | | | | |
| *Celosia argentea* | 54 | 86 K.V. 5 ma. 30 cm. 23 min. | 16 | 15 |
| Portulacaceae | | | | |
| *Calandrinia grandiflora** | 48 | 2500 r-units | 8 | 11 |
| *Portulaca grandiflora* | 41 | 48 K.V. 5 ma. 30 cm. 17 min. | 22 | 24 |
| " " | 27 | 48 K.V. 5 ma. 30 cm. 33 min. | 19 | 21 |
| Ranunculaceae | | | | |
| *Delphinium* | 27 | 87 K.V. 7½ ma. 30 cm. 22 min. | 12 | 4 |
| Brassicaceae | | | | |
| *Cheiranthus** | 15 | 2500 r-units | 14 | 18 |
| *Iberis umbellata* | 74 | 2500 r-units | 16 | 19 |
| *Matthiola bicornis** | 18 | 83 K.V. 5 ma. 30 cm. 22 min. | 9 | 7 |
| Violaceae | | | | |
| *Viola tricolor* | 23 | 85 K.V. 5 ma. 30 cm. 26 min. | 25 | 31 |
| Onagraceae | | | | |
| *Gaura lindheimeri* | 47 | 91 K.V. 5 ma. 30 cm. 20 min. | 9 | 8 |
| Umbelliferae | | | | |
| *Trachymene coerulea* | 24 | 87 K.V. 7½ ma. 30 cm. 22 min. | 23 | 18 |
| " " | 21 | 88 K.V. 5 ma. 29 cm. 17 min. | 9 | 12 |
| Convolvulaceae | | | | |
| *Convolvulus minor** | 21 | 100 K.V. 7½ ma. 30 cm. 22 min. | 11 | 22 |
| *Quamoclit coccinea** | 34 | 83 K.V. 5 ma. 30 cm. 23 min. | 4 | 7 |
| Rubiaceae | | | | |
| *Asperula orientalis** | 51 | 95 K.V. 7½ ma. 30 cm. 25 min. | 11 | 9 |

* Leaf irregularities such as puckering, ligulate form, and mottling due to abnormal chlorophyll development were evident during early growth but disappeared before maturity.

## TABLE I

BY MEDIUM DOSES OF X-RAYS

| Difference in treated plants | | | | | General effects of radiation |
|---|---|---|---|---|---|
| Av. height at maturity | Percentage plants bearing laterals | Av. no. laterals per plant | Av. length of laterals per plant | Age when flowers first appeared | |
| % (av. wt.) | % | % | % | % | |
| − 6.4† | ..... | ..... | ..... | ..... | Greater development of secondary branches |
| +11.5 | − 26.3 | −10.1 | ..... | +0.9 | |
| −38.8 | ..... | −30.8 | +16.5 | ..... | Irradiated plants show shortened inflorescences |
| −25.0 | ..... | ..... | ..... | ..... | Reduction in number of leaves per plant; 82% reduction in plants bearing flower stalks |
| ..... | ..... | Same | +21.9 | ..... | Increased length of branching gives appearance of increased vigor |
| + 8.4 | +172.3 | ..... | +90.0 | +6.7 | No injurious effect apparent |
| −13.8 | None | +25 | ..... | ..... | Retarded growth evident |
| − 6.0 | +210.5 | +34.6 | ..... | ..... | Delay in blossoming and fruiting |
| − 7.8 | ..... | ..... | ..... | +1.3 | Reduction in blossoming; two cases of dichotomous branching |
| − 1.2 | ..... | +73.9 | ..... | ..... | Increase in development of lateral branches |
| + .3 | + 12.9 | +33.3 | ..... | ..... | Decrease in green weight shown in later study |
| −22.2 | ..... | ..... | ..... | ..... | |
| + 7.5 | ..... (Terminal branches) | ..... | ..... | ..... | Greater height probably due to greater development of terminal branches |
| − 3.4 | + 14.9 | ..... | ..... | + 3.4 | |
| −26.7 | ..... | ..... | ..... | − .8 | |
| − 7.2 | ..... | ..... | ..... | −5.3 | |
| −19.6 | − 18.5 | +11.7 | − 7.9 | ..... | |

† In this and other tables in this paper, the minus sign indicates that the treated plants showed less growth than the controls; the plus sign that the treated plants exhibited greater growth.

## TABLE II

GROUP III. SPECIES NOTICEABLY

| FAMILY AND SCIENTIFIC NAME | AGE IN DAYS WHEN IRRAD. | SET-UP FOR DOSE OR DOSE IN R-UNITS | NO. OF PLANTS | |
|---|---|---|---|---|
| | | | CONTROL | IRRAD. |
| Nyctaginaceae | | | | |
| *Abronia umbellata* | 44 | 2500 r-units | 4 | 5 |
| Caryophyllaceae | | | | |
| *Dianthus chinensis* | 21 | 51 K.V. 7½ ma. 30 cm. 22 min. | 21 | 26 |
| " " | 49 | 2500 r-units | 8 | 14 |
| *Gypsophila elegans* | 19 | 50 K.V. 5 ma. 30 cm. 22 min. | 18 | 25 |
| *Lychnis coeli-rosa* | 20 | 44 K.V. 5 ma. 30 cm. 20 min. | 14 | 49 |
| *Lychnis viscaria* | 43 | 53 K.V. 5 ma. 30 cm. 37 min. | 30 | 30 |
| *Saponaria vaccaria* | 8 | 57 K.V. 7½ ma. 30 cm. 22 min. | 11 | 17 |
| *Silene armeria* | 44 | 2500 r-units | 47 | 71 |
| Euphobiaceae | | | | |
| *Euphorbia marginata* | 44 | 2500 r-units | 6 | 5 |
| *Ricinus communis* | ..... | 3500 r-units | 12 | 11 |
| Balsaminaceae | | | | |
| *Impatiens balsamina* | 12 | 50 K.V. 7½ ma. 30 cm. 22 min. | 24 | 45 |
| Malvaceae | | | | |
| *Lavatera splendens rosea* | 19 | 85 K.V. 5 ma. 30 cm. 33 min. | 11 | 15 |
| Onagraceae | | | | |
| *Clarkia elegans* | 27 | 89 K.V. 5 ma. 35 cm. 20 min. | 23 | 16 |
| " " | 27 | 1370 r-units | 12 | 13 |
| " " | 27 | 3750 r-units | 28 | 8 |
| *Godetia amoena* | 20 | 95 K.V. 5 ma. 30 cm. 23 min. | 6 | 5 |

## TABLE II

AFFECTED BY X-RADIATION

| Difference in treated plants | | | | | General effects of radiation |
|---|---|---|---|---|---|
| Av. height at maturity | Percentage plants bearing laterals | Av. no. laterals per plant | Av. length of laterals per plant | Age when flowers first appeared | |
| % | % | % | % | % | |
| −50.7 | − 73.3 | − 66.6 | ..... | + 5.0 | 73.3% reduction in number of flower clusters; 70% reduction in green weight of above-ground parts |
| + 4.5 | + 61.7 | +107.9 | ..... | + 6.3 | Branching greatly increased |
| − 7.1 | ..... | − 34.0 | ..... | −12.4 | 16% reduction in green weight; 12% increase in flower and bud production |
| −16.5 | ..... | ..... | ..... | ..... | Retarded growth; persistent leaf deformities |
| + 7.1 | − 5.1 | Same | +125.3 | ..... | Increase in average length of main branches per plant |
| −21.9 | + 13.4 | + 63.6 | − 11.1 | ..... | Increase in number of lateral branches though not in average length |
| | (Terminal pedicel) | (Pedicels) | ..... | ..... | |
| +13.9 | + 10.1 | + 46.7 | ..... | − 4.0 | Increased height due to greater development of terminal pedicels |
| − 7.3 | + 8.5 | + 33.3 | ..... | + 2.7 | |
| −24.1 | ..... | ..... | ..... | ..... | Leaf deformities conspicuous at maturity; little difference in time of blossoming |
| −18.9 | None | − 21.3 | ..... | ..... | 58% decrease in height to first branch |
| − 5.0 | +216.7 | +237.6 | ..... | + 5.3 | Considerable increase in lateral branching; some dichotomous branching |
| −14.8 | ..... | ..... | ..... | ..... | 70% reduction in leaves at maturity; many deformed leaves present |
| + 9.7 | +140.0 | +103.8 | ..... | + 1.6 | Increased number of lateral branches |
| +77.2 | ..... | + 10.8 | − 1.3 | ..... | |
| +13.6 | ..... | + 45.4 | +103.5 | ..... | Most of irradiated plants died before reaching maturity; resistant ones grew to great size |
| − 2.8 | +200.3 | +800.0 | +706.0 | + 7.5 | Leaves twisted and drawn to one side |

TABLE II—(*Continued*)

GROUP III. SPECIES NOTICEABLY

| FAMILY AND SCIENTIFIC NAME | AGE IN DAYS WHEN IRRAD. | SET-UP FOR DOSE OR DOSE IN R-UNITS | NO. OF PLANTS | |
|---|---|---|---|---|
| | | | CONTROL | IRRAD. |
| Convolvulaceae | | | | |
| *Ipomoea* | 44 | 2500 r-units | 8 | 11 |
| Polemoniaceae | | | | |
| *Cobaea scandens* | 22 | 92 K.V. 7½ ma. 30 cm. 23 min. | 6 | 10 |
| " " | 19 | 93 K.V. 5 ma. 30 cm. 33 min. | 6 | 6 |
| *Gilia* | 18 | 45 K.V. 5 ma. 30 cm. 22 min. | 18 | 23 |
| Hydrophyllaceae | | | | |
| *Phacelia campanularia* | 55 | 2500 r-units | 4 | 4 |
| *Phacelia whitlavia* | 30 | 2500 r-units | 46 | 37 |
| Solanaceae | | | | |
| *Browallia elata* | 39 | 96 K.V. 5 ma. 30 cm. 23 min. | 41 | 51 |
| " " | 52 | 3600 r-units | 31 | 23 |
| *Nicotiana affinis* | 48 | 2500 r-units | 7 | 7 |
| *Schizanthus wisetonensis* | 23 | 95 K.V. 5 ma. 30 cm. 18 min. | 28 | 27 |
| *Salpiglossis sinuata* | 55 | 2500 r-units | 3 | 5 |
| Serophulariaceae | | | | |
| *Alonsa linearis* | 30 | 48 K.V. 5 ma. 30 cm. 22 min. | 22 | 16 |
| " " | 28 | 2750 r-units | 25 | 24 |
| *Antirrhinum majus* | 52 | 2500 r-units | 20 | 23 |
| *Linaria maroccana* | 37 | 105 K.V. 5 ma. 30 cm. 12.2 min. | 16 | 5 |
| *Mimulus tigrinus* | 62 | 2000 r-units | 8 | 8 |

TABLE II—(*Continued*)

AFFECTED BY X-RADIATION

| Difference in treated plants | | | | | General effects of radiation |
|---|---|---|---|---|---|
| Av. height at maturity | Percentage plants bearing laterals | Av. no. laterals per plant | Av. length of laterals per plant | Age when flowers first appeared | |
| % | % | % | % | % | |
| −48.0 | + 63.6 | +180.0 | +200.0 | +20.2 | Leaves showed deformities, light areas, etc., even at maturity; 38% reduction in reproductive structures at conclusion of experiment |
| −30.4 | ..... | ..... | ..... | ..... | Leaf deformities and reduced growth |
| −63.6 | ..... | ..... | ..... | ..... | Leaf deformities still show at maturity |
| − 8.7 | + 14.6 | + 5.9 | + 35.2 | + 4.5 | Greater development of lateral branches |
| | | (Main branches) | | | 2.8% increase in subordinate branches |
| +49.5 | ..... | − 25.9 | − 14.1 | ..... | 2.5% increase in average green weight per plant<br>50% decrease in average number of flowers per plant |
| +10.6 | + 99.0 | + 72.7 | ..... | ..... | Leaves of irradiated plants showed prominent white streaks; greatly increased branching |
| + 5.3 | − 23.1 | −154.0 | ..... | + 7.6 | |
| −78.5 | ..... | ..... | ..... | ..... | Blossoms never appeared, even after 122 days when controls were fruiting; irradiated remained dwarfed |
| −29.4 | ..... | ..... | ..... | ..... | Retarded growth; leaf deformities; reduced blossoming |
| −38.0 | ..... | ..... | ..... | +11.1 | 47% reduction in number of inflorescences; 25% reduction in length of inflorescences |
| − 7.5 | ..... | ..... | ..... | +16.2 | Over 50% reduction in buds and flowers |
| + 6.5 | + 37.4 | +137.6 | +141.1 | −18.1 | Some dichotomous branching |
| − 9.9 | Each 100% | − 9.6 | ..... | ..... | 33% of irradiated plants showed dichotomous branching |
| −10.3 | + 20.5 | + 5.5 | + 8.2 | ..... | Increased branching; reduced height; leaves in early stages showed spotting and streaking |
| −30.8 | − 80.0 | ..... | ..... | ..... | Great increase in number of main branches from the base; fasciation in one central stalk |
| −31.7 | ..... | − 22.2 | ..... | ..... | Reduced height; flowers of irradiated plants appear lighter in color |

TABLE II—(*Continued*)

GROUP III. SPECIES NOTICEABLY

| FAMILY AND SCIENTIFIC NAME | AGE IN DAYS WHEN IRRAD. | SET-UP FOR DOSE OR DOSE IN R-UNITS | NO. OF PLANTS | |
|---|---|---|---|---|
| | | | CONTROL | IRRAD. |
| Scrophulariaceae | | | | |
| *Nemesia versicolor* | 14 | 57 K. V. 7½ ma. 30 cm. 23 min. | 5 | 18 |
| Campanulaceae | | | | |
| *Campanula medium* | 47 | 2650 r-units | 30 | 8 |
| Valerianaceae | | | | |
| *Centranthus macrosiphon* | 44 | 2500 r-units | 3 | 16 |
| Carduaceae | | | | |
| *Acroclinium roseum* | 20 | 100 K.V. 5 ma. 30 cm. 23 min. | 16 | 11 |
| " " | 27 | 3000 r-units | 13 | 12 |
| *Arctosis stoechadifolia* | 29 | 50 K.V. 5 ma. 30 cm. 22 min. | 10 | 16 |
| *Brachycome iberidifolia* | 44 | 2500 r-units | 54 | 85 |
| *Dimorphotheca aurantiaca* | 22 | 93 K.V. 5 ma. 30 cm. 25 min. | 12 | 5 |
| " " | 12 | 2500 r-units | 24 | 29 |
| *Leptosyne stillmanii* | 8 | 91 K.V. 7½ ma. 30 cm. 22 min. | 12 | 11 |
| *Rhodanthe manglesii* | 26 | 90 K.V. 5 ma. 29 cm. 17 min. | 58 | 41 |
| *Sanvitalia procumbens* | 48 | 2500 r-units | 16 | 47 |
| Cichoriaceae | | | | |
| *Centaurea americana* | 49 | 2500 r-units | 5 | 2 |

TABLE II—(*Continued*)

AFFECTED BY X-RADIATION

| DIFFERENCE IN TREATED PLANTS | | | | | GENERAL EFFECTS OF RADIATION |
|---|---|---|---|---|---|
| AV. HEIGHT AT MATURITY | PERCENTAGE PLANTS BEARING LATERALS | AV. NO. LATERALS PER PLANT | AV. LENGTH OF LATERALS PER PLANT | AGE WHEN FLOWERS FIRST APPEARED | |
| % | % | % | % | % | |
| −12.4 | − 7.5 | − 6.2 | ..... | − 2.4 | Leaf irregularities, ligulate form, pebbly, etc. |
| −45.7 | ..... | ..... | ..... | ..... | Dose lethal to many seedlings; at close of the experiments when many controls were blossoming, 15.5% more of irradiated than controls were still in rosette form. Average green weight of irradiated plants was 40% less than that of controls |
| −15.8 | −62.4 | −83.7 | −80.3 | − 2.5 | Puckered leaves; irregular chlorophyll development |
| +11.2 | ..... | +39.0 | ..... | +15.0 | Apparent increase in vegetative growth |
| −39.8 | ..... | +22.2 | − 1.4 | ..... | Blossoming delayed; number of main branches increased |
| −11.6 | −43.8 | −15.0 | ..... | ..... | Leaf irregularities; terminal as well as lateral growth checked |
| − 6.8 | −14.0 | −57.1 | ..... | +17.6 | Blossoms decreased 65%; 43% decrease in green weight |
| +30.6 | None | + 4.2 | ..... | ..... | Increased height and branching |
| +12.4 | ..... | −14.8 | −12.5 | ..... | Some increase in average number of subordinate branches per plant, but 27% decrease in average length per plant |
| −28.4 | ..... | ..... | ..... | − 3.5 | One case of dichotomous branching |
| −16.9 | −26.7 | −16.7 | ..... | + 7.3 | Reduction in total growth and branching |
| −34.8 | − 5.1 | −27.2 | −50.0 | − 4.6 | 33% reduction in average number of flowers; ligulate flowers interspersed among disk flowers |
| −10.6 | ..... | ..... | ..... | +10.8 | Plants noticeably injured; deformed, discolored leaves; dichotomous branching from base |

rather good recovery, so that at maturity it was difficult to judge results without careful measurements and calculation of the percentage difference in the treated plants. All measurements taken and counts made are available for comparisons, but only calculations of percentage differences in treated plants are given in tables I and II. In a few cases the number of plants grown to maturity falls below a dozen specimens because of difficulties encountered in growing some of these forms in the greenhouse. Since even a small number of plants may give some indication of the susceptibility of the species, results involving a relatively small number of specimens have been included, in a few cases, with the idea that the reader will accept the results for these few species as being preliminary in nature.

The age in days at the time of irradiation necessarily shows considerable variation in range because of differences in germination and growth response of the various species. Treatment was given when the true foliage leaves were just beginning their development.

The following species in this group during early growth showed irregularities in the first few leaves which developed after radiation: *Chenopodium album, Spinacia oleracea, Calandrinia grandiflora, Cheiranthus, Matthiola bicornis, Quamoclit coccinea,* and *Asperula orientalis.* In some cases the leaves were deformed, puckered, and twisted to one side; in others a greater or less degree of mottling indicated some disturbance in chlorophyll development.

Particular attention is called to certain points indicated in table I. *Spinacia* and *Portulaca* are the only species which showed an increase in the average height. Although *Trachymene* is listed as showing the irradiated plants with greater height, this is probably due to a better development of terminal branches rather than to the development of the central stalk. Three species, *Cheiranthus, Viola,* and *Portulaca,* had a greater percentage of irradiated specimens than controls bearing lateral branches. Experimental plants of *Delphinium, Cheiranthus, Matthiola,* and *Viola* showed an increase in average number of lateral branches per plant. This condition commonly accompanies x-radiation, as shown by ANCEL (**1**) and the writer (**5, 7, 9, 10**). In *Celosia argentea,* there occurred a reduced number of lateral branches, but an increase in their average length. In *Asperula orientalis,* on the other hand, while there was an increase in the average number of lateral branches there was a decrease in their average length. In general, it has been found (**7, 9, 12**) that irradiation causes a delay in the time of blossoming. Delay is not as apparent in this group as in the third group.

### GROUP III. SPECIES NOTICEABLY AFFECTED BY X-RADIATION

The dose of 2500 r-units, which was used on most of the species listed, proved lethal to seedlings of *Nemophila insignis, Gaillardia picta,* and *Phlox*

*drummondii*. A study with *Nemophila*, where the effects of various doses were determined, is reported elsewhere (**12**).

Forms which showed effects of treatment during the early seedling stages, and which at maturity showed also changes such as difference in height, number, and length of branches, and unusual cases of dichotomous branching, are grouped together in table II. The same general plan was followed with these species as was used with those of the other group. In general, a medium dose depresses the total growth when height of the plant is used as an indicator of growth. Of the thirty-nine species listed, but nine of them showed any increase in the total height of the treated plants.

The experimental plants of *Dimorphotheca* and *Clarkia* showed increase in height in several experiments. With the former, although the experimental plants increased in average height, there was a slight decrease in green weight. In *Clarkia* the dose was lethal for a considerable number of plants, and those remaining proved to be exceptionally strong, vigorous plants. This may also have been true for treated plants of *Acroclinium*, *Phacelia*, and *Saponaria*, which gave significant increases in total height.

The studies of others (**2, 3, 4, 13, 14, 16, 17**) and former experiments by the writer (**5, 7, 9**) have demonstrated the tendency for leaves of x-rayed plants to exhibit irregularities in shape as well as a peculiar pebbly, blotched appearance of the blade. Species differ considerably with respect to the length of time that this effect is evident. Nearly all the plants listed in this group had leaf deformities in their early growth stages. Those species showing less conspicuous deformities include *Gilia*, *Alonsa*, *Browallia*, *Mimulus*, and *Rhodanthe*.

X-radiation of some species (**5, 7, 9, 10**) is commonly accompanied not only by an increase in the percentage of plants bearing laterals but also by an increase in the number and average length of lateral branches per plant. Some species which normally do not form lateral branches, after irradiation often show either dichotomous or lateral branching. In one-third of the species listed in table II, the irradiated plants showed an increase over the controls in the percentage of plants bearing lateral branches. The increased branching accompanying radiation was most noticeable in members of the Caryophyllaceae and in the genera *Impatiens*, *Clarkia*, *Godetia*, *Ipomoea*, *Gilia*, *Phacelia*, *Antirrhinum*, and *Acroclinium*.

The time of first appearance of flowers was delayed in all species of experimental plants except six. Dichotomous branching, so common in irradiated *Helianthus* and *Zinnia*, appeared also in *Impatiens*, *Alonsa*, *Centaurea*, and *Leptosyne*.

### *Dimorphotheca aurantiaca*

The effect of treatment upon the cape marigold, *Dimorphotheca aurantiaca*, of the Carduaceae is discussed to illustrate the fact that not all plant

species react in exactly the same manner to radiation. This species appeared severely affected during its early stages of growth but treated plants later showed increased height over the controls. After a preliminary experiment had indicated the sensitivity of this species to x-radiation, two more groups of plants were grown in which the seedlings were given approximately 2500 r-units. The leaves showed the usual effects, for they were irregularly ligulate in shape and presented the characteristic pitted, blotched appearance. The total height of the first group was increased 30.6 per cent. but there was little difference in the number of branches or in the time of blossoming. The increase in height was such an unusual response to radiation that a larger group of plants was grown and more complete measurements were taken.

Table II gives the percentage differences between the growth of control seedlings and those given 2500 r-units when twelve days old. In this group, growth of the treated seedlings was retarded for a time but at the age of five months, when the experiment was concluded, the average height of the central stalk was somewhat greater in the treated plants. The number of main lateral branches in the treated plants was decreased; in the subordinate branches, which developed later, the number was increased 9 per cent. but their average length per plant decreased 27 per cent. The average green weight of the treated plants, however, was 5 per cent. less than that of the controls, indicating that although these plants appeared to show increased size, there really was no actual increase in amount of plant tissue produced.

Counts of the number of faded blossoms as well as of the fresh flowers and buds were made at the conclusion of the experiment. Calculations of the percentage of the total number which were living at the end of five months showed 37 per cent. decrease on the main lateral branches of irradiated plants, but 228 per cent. increase on the central stalk, and a slight increase in the number of those on the subordinate branches. The greater percentage of living flowers and buds was present on the more recent vegetative growth of the treated plants because in the controls more of the flowers were older and had faded. The retarded vegetative growth was thus accompanied by delayed flowering.

This species does not show injury due to irradiation as clearly as do many of the composites. Although growth is retarded during the earlier stages, the experimental plants attained to as great or greater height than did the controls. The average green weight of the treated plants, however, was slightly less than that of the controls. The number of branches was in general decreased. The number of flowers produced was little affected by treatment, although the irradiated plants showed a greater number of living flowers at the close of the experiment because of the fading of the early flowers of the control.

### *Linaria maroccana*

*Linaria maroccana* of the Scrophulariaceae showed a noticeable increase in the number of main branches from the base other than from the central stalk. This is a good example of a plant which from the first had difficulty in recovering from the x-ray dose. Although the leaves showed little effect, the stems were weak and decumbent, and at an early date an increase in branching was apparent. Growth habits in the treated plants were considerably altered. They had a thickened, twisted, hypocotyl region, and little distinction between main and secondary stalks. Table II shows the reduced height and decrease in lateral branches in the rayed plants. No flowers developed in the treated plants.

### *Campanula medium*

*Campanula medium* seedlings showed a heavy mortality when given 2650 r-units. Of those which survived, 15 per cent. more of the irradiated than

FIG. 1. *Campanula medium* plants 7 months old: Left, controls; right, plants irradiated with 2650 r-units. Plants which survived the dose showed greatly reduced height and weight. None of treated plants had blossomed at conclusion of experiment.

controls were still in the rosette stage when 208 days old. The controls were rather crowded, hence many remained in the rosette form that might otherwise have formed shoots. Table II records the lessened height and green weight of the treated plants. Figure 1 represents typical members of the control and treated groups at the close of the study. None of the experimental plants had blossomed at the conclusion of the experiment.

### *Salpiglossis sinuata*

*Salpiglossis sinuata* proved to be ray susceptible as are many other members of the Solanaceae. Figure 2 represents the difference between irradiated

FIG. 2. *Salpiglossis sinuata* plants 160 days old: Left, controls; right, plants which received dose of 2500 r-units in seedling stage. When the photograph was taken, the average height of the irradiated plants was 57 per cent. less than that of the controls; the average number of buds and flowers was 5.3, while none had appeared in the treated plants.

and control plants when about five months old. Later, the irradiated plants showed more rapid growth so that when the records given in table II were taken, there was but 7.5 per cent. difference in average height between the control and the experimental plants. Flowering was greatly delayed and reduced in the treated plants.

### *Ricinus communis*

*Ricinus communis* when irradiated with 3500 r-units showed reduction in average height due to a 58 per cent. decrease in average height to the first branch. This confirms the statement of RIVERA (**15**) that terminal shoot development of the castor bean is arrested by x-radiation. Figure 3, which

FIG. 3. *Ricinus communis* plants 8 months old: Left, controls; right, plants treated with 3500 r-units in seedling stage. The latter show reduced total height and increased branching. Most noticeable difference is 58 per cent. reduction in average height to first branch.

represents typical plants 222 days after treatment, shows the shortened stem below the branches as well as the reduced height. A mottled appearance of the leaves, decrease in average number of branches, and a reduction in number and length of roots are manifest in this species as in others.

### DAY LENGTH AS INFLUENCING ACTION OF RAYS

The greater number of plants experimented upon were planted in the autumn, irradiated during the short days of winter, and records concerning total growth and branching were taken at maturity which occurred in the spring or early summer. Two species of plants, *Limonium sinuatum* and *Thunbergia alata,* which were irradiated during the long days of spring rather than during the short days of winter, gave unusual results which need special discussion.

*Limonium sinuatum.*—To determine whether *Limonium sinuatum,* a member of the Plumbaginaceae, was affected by the rays in a different way than are the majority of plants, it was grown three different years. Table III summarizes observations of inflorescence development of the members of the three groups. Seedlings of the first group, 18 days old, were irradiated in

## TABLE III

EFFECT OF X-RADIATION UPON INFLORESCENCE DEVELOPMENT OF *LIMONIUM SINUATUM*

| GROUP NO. | DATE OF TREATMENT | AGE IN DAYS WHEN IRRADIATED | DOSE | NO. OF PLANTS | | DIFFERENCE IN TREATED PLANTS | |
|---|---|---|---|---|---|---|---|
| | | | | CONTROL | IRRADIATED | AV. HEIGHT OF INFLORESCENCE | AV. NO. OF MAIN BRANCHES OF INFLORESCENCE |
| I | April 16 | 18 | 48 K.V. 5 ma. 30 cm. 33 min. | 8 | 11 | % None in irrad. | % None in irrad. |
| II | (1) Nov. 20 | 15 | 93 K.V. 5 ma. 30 cm. 23 min. | 16 | 12 | +3.5 | +44.4 |
| | (2) Dec. 13 | 38 | 90 K.V. 5 ma. 30 cm. 15 min. | | | | |
| III | Dec. 6 | 24 | 80 K.V. 5 ma. 30 cm. 29½ min. | 10 | 5* | −1.0 | + 6.2 |

* During transplanting 50 per cent. of treated plants died.

April with one dose from the following set-up: 48 K.V. 5 ma. 30 cm. for 33 minutes. Eight months later when the study was concluded, 75 per cent. of the controls bore inflorescences while all the experimental plants remained in the vegetative stage. Leaves of the treated plants remained vegetative and were larger and more vigorous than those of the controls although there was little difference in the average number per plant. Roots of the control plants were long and spindling as compared with the short stocky growth in the other group.

The development of the inflorescences in the control and its absence in the irradiated plants constituted the most striking difference between the two groups. Because of the total absence of inflorescences in the treated group, other experiments were made to determine whether irradiation always prevented blossoming of members of this genus.

Seedlings of the second group which were irradiated in November were given two doses, the first from the set-up 93 K.V. 5 ma. 30 cm. for 23 minutes. After an interval of two weeks, a second dose of slightly less intensity was given. The irradiated plants were retarded in growth, had twisted and ligulate leaves, and, when at the age of four months the controls began to send up flowering stalks, the irradiated plants still remained in the vegetative

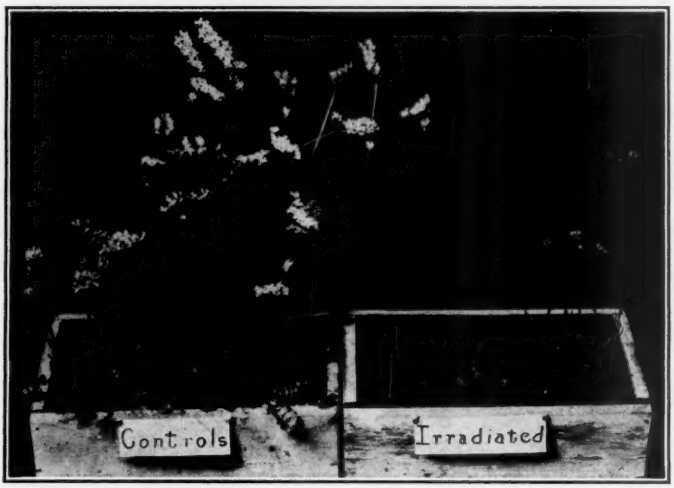


FIG. 4. *Limonium sinuatum* plants (group II) 6 months old. Seedlings of those at right were given two doses of approximately 1000 r-units in November. Note reduced vegetative and reproductive growth of treated plants. Plants of group I (not shown here) irradiated in April were still in rosette stage after 8 months; at this time, 75 per cent. of the controls bore inflorescences.

condition. Figure 4, taken at the age of six months, shows representative plants from both the control and irradiated groups. This experiment differs from the one previously described in that the vegetative and reproductive growth of the treated plants was greatly retarded, but flowering was not completely inhibited. Ten months after planting, the controls had all matured and were entirely dead while the green experimental plants showed stalks of the inflorescence which averaged as tall as those of the controls and they had 44 per cent. increase in the average number of flower stalks.

The following December, seedlings of another set, group III, were given one dose with the set-up 80 K.V. 5 ma. 30 cm. for 29½ minutes. Many of these died during transplanting, for their vitality seemed greatly lowered by the treatment. The vegetative portion of those remaining continued to be small and deformed but when the inflorescence finally developed, as many scapes were produced by the irradiated plants as by the controls and finally the branches of the inflorescence of the treated specimens attained practically the same height as the control plants.

*Limonium* which was irradiated during the fall reacted in about the same manner as other ray-sensitive species. The two groups of plants which were grown during the winter and spring developed normal inflorescences although they were considerably delayed. Plants germinated and irradiated in April, on the other hand, remained vegetative and at the end of eight

TABLE IV

EFFECTS OF X-RADIATION UPON *THUNBERGIA ALATA*

| RECORDED DATA | GROUP I* (GROWN DURING SHORT DAYS) | | | GROUP II* (GROWN DURING LONG DAYS) | | |
|---|---|---|---|---|---|---|
| | CONTROL | IRRADIATED 1280 R-UNITS | DIFFERENCE IN TREATED PLANTS | CONTROL | IRRADIATED 1320 R-UNITS | DIFFERENCE IN TREATED PLANTS |
| | | | % | | | % |
| No. of plants | 15.0 | 12.0 | ..... | 16.0 | 21.0 | ..... |
| Av. length of central stalk (cm.) | 18.7 | 16.4 | −12.3 | 10.7 | 9.9 | − 7.5 |
| Av. number of lateral branches per plant | 3.5 | 2.7 | −22.8 | 2.6 | 2.0 | −23.1 |
| Av. length of lateral branches (cm.) | 25.0 | 12.2 | −51.2 | 12.4 | 14.0 | +12.9 |
| Av. length of lateral branches per plant (cm.) | 87.0 | 33.6 | −61.4 | 32.5 | 28.7 | −11.7 |
| Av. time (days) before blossoming occurs (Data taken for each pot; not for each plant) | 103.8 | 111.5 | + 7.41 | 94.4 | 100.5 | + 6.5 |

* Seeds for group I planted Nov. 5, 1931; seeds for group II planted Jan. 22, 1932.

months had not formed inflorescences. The controls blossomed at the usual time.

*Thunbergia alata.*—A member of the Acanthaceae, *Thunbergia alata,* showed greater injury to the vegetative growth and increased retardation in flowering when irradiation was given during the period of short days rather than when it occurred later in the spring. Seeds of group I were planted during the first week of November, and blossoming began the middle of February. Seeds of group II were planted the last of January, and blossoms first appeared the middle of April. The plants of group I were thus exposed to shorter days than were those of group II.

The leaves of all the experimental plants showed marked irregularities in shape, color, and size. Table IV indicates that length of central stalk, average length of lateral branches per plant, and average time before blossoming occurs, were very considerably reduced in all irradiated plants of both groups. Those planted and irradiated in the fall exhibited greater retardation in all these respects than did those planted and irradiated in the late winter and spring.

## Discussion

The 15 species listed in group I (those apparently unaffected by x-rays) were judged chiefly by the presence or absence of leaf irregularities, average height at maturity, and time of blossoming—in other words, by visible symptoms. Had green and dry weight determinations been made, there is a possibility that all irradiated plants would have shown some reduced growth, for the stonecrop (*Sedum*) and ice plant (*Mesembryanthemum*), although manifesting no apparent injury, were found to have reduced green weight.

Difficulty arose in some cases in classifying plants with relation to x-ray susceptibility. Decision was based principally on the duration of leaf anomalies, if any occurred, as well as upon average height and branching. Other observers might have made somewhat different groupings.

Very few of the forty species listed among those which were noticeably affected developed any increase in total height of treated plants. In one-third of the group, among the irradiated there was a larger percentage of plants bearing lateral branches. Members of the Caryophyllaceae and Onagraceae were thus influenced. Dichotomous branching, which occurs so frequently in irradiated sunflower and zinnia plants, appeared in a few other species, particularly among composites.

In the severely affected group the first appearance of flowers was delayed, and usually the percentage of plants bearing flowers was reduced.

Studies with plants such as the cape marigold (*Dimorphotheca*) show the necessity of repeating experiments and waiting until maturity before drawing conclusions. This species manifested remarkable ability to recover

from the effects of the rays. Although there were fewer main lateral branches produced, the subordinate branches were more numerous and of greater average length. The blossoming on the central stalk and on the subordinate branches was considerably delayed in the treated plants although it was not actually decreased.

Other plants cited, because of their response to treatment, include: *Linaria,* which showed notable increase in the number of main branches from the crown; *Campanula,* typical of those plants which when irradiated remain for a long time in the rosette stage; and *Ricinus,* in which the branching occurred very much nearer the base of the plant in treated specimens.

Opportunity to observe how length of day affected x-rayed plants was presented in the case of *Limonium sinuatum* and *Thunbergia alata.* Plants of the former species rayed in the spring remained vegetative and in the rosette stage for eight months, while controls blossomed as usual. Irradiation in autumn brought about delay in blossoming but not complete inhibition. *Thunbergia* treated in the autumn was much more severely affected than were seedlings of the same species irradiated in the spring. It is possible that all species may be more injured when their seedlings are treated during short days.

It is impossible at this time accurately to state reasons for resistance or susceptibility of certain species, but observations from the large number of species examined seem to establish the fact that members of Chenopodiaceae, Portulacaceae, and Brassicaceae are in general resistant. The following genera in widely separated families, suffered some change in growth form, but on the whole were not noticeably injured: *Impatiens, Clarkia, Godetia, Gilia, Alonsa, Antirrhinum, Phacelia,* and various members of the Caryophyllaceae. Members of Solanaceae, Scrophulariaceae, and Carduaceae were the most severely affected. Genera noticeably ray-susceptible are *Abronia, Ricinus, Lavatera, Ipomoea, Cobaea, Linaria, Campanula, Centranthus,* and *Thunbergia.*

## Summary

1. The seventy species of flowering plants distributed in thirty-five families considered in this paper were grouped as follows: fifteen species apparently unaffected by the rays; fifteen slightly affected, particularly during their early growth stages; the remaining forty species noticeably affected.

2. Members of the Chenopodiaceae, Umbelliferae, and Brassicaceae often gave indications of injury soon after treatment but by the time maturity was reached, manifested little effect.

3. Plants noticeably injured by radiation were marked by decrease in total height; increased branching as in the Caryophyllaceae and Onagraceae; frequent occurrence of dichotomous branching, particularly in the Cardua-

ceae; irregularities in shape, margins, and chlorophyll development of the leaves; delayed and reduced blossoming.

4. Members of the Solanaceae, Scrophulariaceae, and Carduaceae were noticeably ray-susceptible, as were also the following genera in other families: *Abronia, Ricinus, Lavatera, Ipomoea, Cobaea, Linaria, Thunbergia, Campanula,* and *Centranthus.*

Acknowledgment is made to the Committee on the Effects of Radiation upon Living Organisms of the National Research Council for assistance in making this study, and to the Physics Department of the University of Colorado for the use of the x-ray machine. Thanks are extended to Professor FRANCIS RAMALEY for helpful criticism of the manuscript.

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# LEAF TEMPERATURES AND THE COOLING OF LEAVES BY RADIATION

OTIS F. CURTIS
(WITH SEVEN FIGURES)

There has been marked disagreement among plant physiologists relative to the effectiveness and importance of transpiration in cooling the leaves of plants. SMITH (**12**), CLUM (**4**), CURTIS (**5**), and others have concluded that the cooling effect is slight and rarely of importance in preventing excess heating, whereas, SHULL (**10**), EATON and BELDEN (**7**), ARTHUR and STEWART (**1**), CLEMENTS (**3**), and others have concluded that the cooling effect may be great. The latter investigators especially have claimed that the cooling effect is much greater than is commonly recognized. ARTHUR and STEWART, and CLEMENTS have claimed that the excessively high temperature of leaves, when they are inclosed in cellophane envelopes, or in glass chambers and exposed to strong light, is due to the fact that the cellophane or the glass effectively stops transpiration; while the failure of wilted, or vaselined leaves to rise in temperature more than 2° to 5° C. above those not so treated is due to the ineffectiveness of these treatments in preventing transpiration. As the writer pointed out in a recent paper (**6**), however, these investigators overlooked the fact that glass and cellophane are heat traps permitting the passage of visible radiation, but preventing much of the loss of heat by interfering with cooling by air currents, as well as by reducing loss by radiation in the infra-red. It was found that dry black paper showed similar marked rises in temperature when inclosed in cellophane envelopes, and in this case evaporation could play no part.

Although the effects of various factors upon leaf temperatures have been investigated by several workers, almost no attention has been given to the loss of heat by radiation in the infra-red. BROWN and ESCOMBE (**2**) speak of loss of heat by emission, and include loss by both conduction and radiation. In their preliminary discussion of a hypothetical case they state that the walls of the inclosure must be at the same temperature as the inclosed air, but, in the actual experiments cited, no mention is made of the wall temperature, and it is assumed that the air temperature is the same as that of the leaf's surroundings. It is also assumed that if the air temperature is equal to or exceeds that of the leaf, there will be no loss of heat from the leaf by radiation. They, WATSON (**13**), and other investigators seem to have failed to realize, that the oxygen and nitrogen of the atmosphere are almost transparent to radiation in the infra-red, and that in such an atmosphere leaves may become cooler than the air about them, owing to radiation

to cooler objects, or to space at a distance; or warmer owing to receipt of radiant energy from them.

It is well known that on a clear night leaves or other objects at or near the surface of the earth may become cooler than the air about them because of radiation. Under these conditions dew or frost is likely to form on them. It is also well known that clouds will act as a blanket, largely preventing such loss of heat by radiation. The maximum radiation at earth temperature (293° C. absolute) occurs at wave lengths of about 10 μ. Although water vapor has a band of relative transparency at about 10 μ, for the range from 1.4 to 20 μ, water is almost opaque, more so than any other known substance. This accounts for the blanketing effect of clouds. At night they radiate (or reflect) back to the earth much of the infra-red that is radiated to them. In the daytime they absorb some infra-red directly from the sun, part of which also is radiated to objects at the earth's surface.

This effect of clouds led the writer to believe that possibly the low temperature for leaves in sunlight, which EATON and BELDEN (7) reported for cotton in Arizona, might be accounted for by the fact that these measurements were made in a region with very low humidity, for it has been found by FOWLE (8) that water vapor has strong absorption bands in much the same region of infra-red as has liquid water.

## Experimentation

In order to test the effectiveness of radiation in cooling leaves, a preliminary experiment was carried out with bits of black paper 1 cm. square exposed to radiant energy in a chamber where the gas in the chamber, and the temperature of the chamber walls, could be controlled.

The chamber consisted of a thin walled, cylindrical, tinned, and blackened tank, 18 cm. in diameter and 28 cm. deep. It was submerged in a large water bath so that only about a centimeter of the upper rim extended above the water. This water bath served to keep the lateral walls and the bottom of the chamber at approximately constant temperature. Radiant energy from a 500-watt lamp, placed at a distance of about 50 cm., entered from above through a circular opening 7.5 cm. in diameter. In the experiment a single sheet of black paper, which is somewhat transparent to infra-red, and itself radiates when heated by visible radiation, was clamped over the circular opening so as to prevent loss of the contained gases.

Thermocouples were held in position at different levels in the chamber by tying them to threads stretched between two wooden rods projecting vertically downward from the cover. Strips of black needle paper, 22 mm. long and 10 mm. wide, were folded double and one edge folded over about 2 mm. to hold the edges together. These squares of black paper were slipped

over the thermocouples and so adjusted as to be exposed at right angles to the incident radiation.

For making the temperature readings, thermocouples made of copper and constantan wire of no. 30 gauge were used. (No. 36 gauge wires would have been preferable, especially for thin leaves.) The standard junction was placed in a thermos bottle filled with ice and distilled water. The equipment used consisted of a Leeds and Northrup Type K-2 potentiometer, a portable galvanometer (no. 2420B), a standard cell, and two ordinary 1.5-volt dry cells. These were all mounted in a shallow box which could be carried into the field. With this outfit temperature can be read to about 1/20° C., but no attempt was made to read closer than 1/10° C., because leaves often change in temperature more than fifty times this amount in 10 to 15 seconds. For making leaf temperature readings the thermocouple wires, which extended about a centimeter beyond the insulation, were threaded through the leaf so that the thermocouple junctions as well as that part of the wires back to the insulation would touch the leaf surface. I find this method far superior to that of MILLER and SAUNDERS (9), or of EATON and BELDEN (7) for following changes in specific leaves. A comparison of the methods will be discussed later.

In measuring the temperature of the paper squares placed at different levels in the chamber and exposed to radiant energy, it was found that there were no differences in temperature in dry and nearly saturated air. It immediately became apparent that this was due to the fact that too little water vapor was present in so small a chamber to have any marked effect. At the temperatures used, the chamber could contain only 20 to 30 mg. of water vapor per liter. A depth of only about 9 cm. of gas separated the thermocouples from the walls. A gas was then sought which could be used in much higher concentrations than water vapor, and which also had strong absorption in the infra-red. Carbon dioxide was found to be suitable. The experiment was therefore repeated with the chamber filled with $CO_2$ at atmospheric pressure in order to compare its effect with air. Curves showing readings of such an experiment are presented in figure 1.

The temperature of the water bath rose slightly during the experiment so that the second set of readings for the bath, although parallel to the first set when $CO_2$ was in the chamber, was somewhat higher. To correct for this, the experiment was repeated in the reverse order, that is, air was used first, followed by $CO_2$. Similar differences were found, but greater by the amount of difference in the bath temperatures. Except for this slight difference both sets of curves were exactly the same.

The paper squares became warmer by about 3° C. in the atmosphere of $CO_2$ than they did in air. This is probably due to the fact that the $CO_2$ is absorbing some of the infra-red coming directly from the lamp as well as

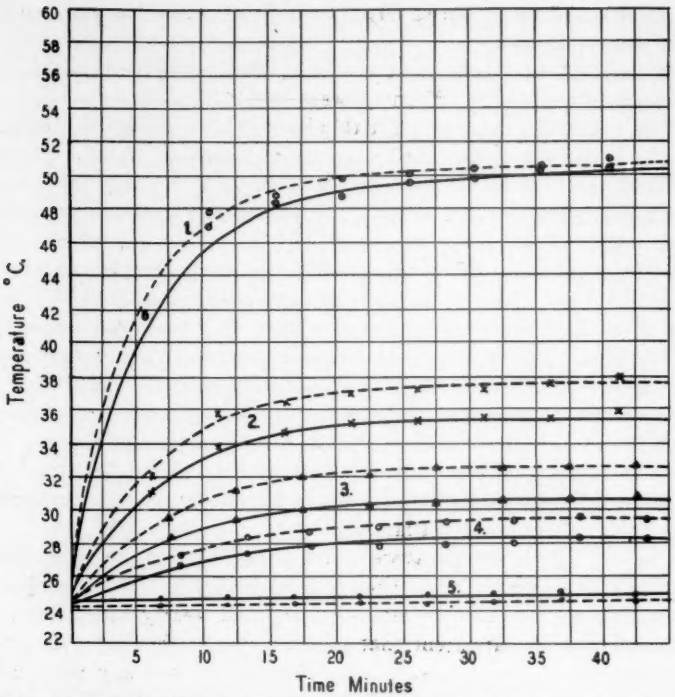


FIG. 1. Curves showing the temperatures of squares of black paper in an atmosphere of $CO_2$ as compared with one of air. Solid lines represent temperatures in air; broken lines, temperatures in $CO_2$. No. *1*, thermocouple touching black paper closing opening of chamber; *2*, square in chamber 1 cm. from paper at top; *3*, square in chamber 2 cm. from paper at top; *4*, square in chamber 5 cm. from paper at top; *5*, temperature of bath in which chamber was submerged.

that radiated to it from the paper squares, while the air, being transparent, does not act as a warming blanket but allows the heat to be radiated more easily to the walls of the chamber. It is recognized that the rate of heat removal from the squares by conductance would be slightly less in $CO_2$ than in air because the relative heat conductance of $CO_2$ is about 0.6 that of air, but estimates indicate that this would account for less than one per cent. of the difference obtained.

In order to test the effectiveness of heat loss from leaves by radiation in the infra-red, a simple apparatus was set up by which a cold surface could be placed near the leaves.[1] A narrow tank 6.5 cm. wide, 41 cm. deep, and

[1] This part of the investigation was performed at the University of California at Berkeley where facilities were supplied by the Division of Plant Nutrition.

41 cm. long was made of galvanized iron. In the center of the two opposite faces a hole 5 cm. in diameter was cut. These holes accommodated rubber stoppers through which glass tubes 3 cm. in diameter and 10 cm. long were inserted. All sides of the tank were insulated by sheets of "celotex" 1 cm. thick. One face of the metal box was blackened with a mixture of lamp black and varnish in order to make a good absorbing or radiating surface. On this face the sheet of celotex was so adjusted as to be easily removable. A microscope lamp placed at the tube opening at the back of the box served to supply the radiant energy. In operation, the box was filled with crushed ice and water. The cold blackened surface of the box represents a clear sky; the lamp, the sun; and the movable celotex shield, a cloud or atmosphere rich in water vapor.

In a representative experiment cut leaves of *Coprosma baueri* with thermocouples inserted in them were placed 23 cm. from the front face of the cold box. One leaf was so placed that it would receive light through the opening in the cold box, while another was placed a few centimeters to one side so as to receive no light. A bare thermocouple for measuring the air temperature was placed at the same distance from the box but was shielded from it by a small square of celotex $2 \times 2$ cm. placed about 2 cm. from the thermocouple. A second bare thermocouple was placed at the same distance from the cold box, but not shielded from it. Temperature readings were made at 5-minute intervals. In this series the temperatures of six couples were recorded. Two of the curves, one for air temperature, and the other for a second leaf are not included in the figure as they ran parallel to those here given. It took about 2 minutes to read the six temperatures. Although the time interval for any given thermocouple was 5 minutes, because they were always read in the same order, the actual time of reading did not coincide for the different thermocouples. To eliminate difficulties in drawing the curves all points in these and subsequent curves are placed as if all were taken at the same time. The data are presented in figure 2.

In this experiment the front face of the cold box was covered at first by the celotex shield. At point *1* the light was turned on; at point *2* the door of the laboratory was opened and the draft of cold air caused a temporary drop in temperature of all the thermocouples. At point *3* the door was opened again with the same results. At point *4* the celotex shield was removed from the cold box, which resulted in a fall in temperature of both lighted and shaded leaves, a slight drop in the bare thermocouple which could radiate to the cold surface, but no change in the air temperature as indicated by the bare thermocouple which was shielded from the cold box. At point *5* the shield was replaced on the face of the cold box, which resulted in an abrupt rise of leaf temperature accompanied by only a slight and gradual rise in air temperature. At point *6* the door was again opened, re-

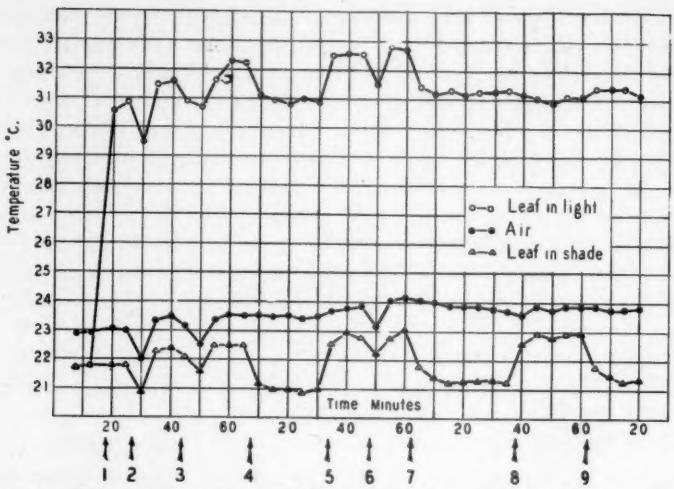


FIG. 2. Cooling of leaves by radiation to a cold box: point *1*, light was turned on and leaf receiving the light rose in temperature; *2*, *3*, and *6*, door of the laboratory was opened, and cool air lowered the readings for all thermocouples; *4*, shield was removed from cold box. (Note drop in temperature of leaves but not the air); *5*, shield replaced on cold box; *7*, shield removed; *8*, large sheet of glass shielded the cold box; *9*, glass removed.

sulting in a cooling draft of air. At point *7* the shield was again removed, with the resultant sudden drop in leaf temperature. The slow drop in air temperature was probably due to the loss of heat from the many surrounding objects by radiation to the cold box, and the slight cooling of the air by conduction. At point *8* a large sheet of glass was placed in front of the cold box in place of the celotex shield. The temperature of the leaf in the light dropped slightly, while the temperature of the leaf in the shade rose abruptly. This rise in temperature of the shaded leaf is undoubtedly due to the failure to lose heat to the cold box by radiation. In like manner the glass must have prevented loss by radiation from the leaf in the light, but to an approximately equal extent it also reduced the radiant energy reaching the leaf from the light, for there was no hole through the glass shield as there was through the celotex shield. At point *9* the glass was removed and the temperature of the leaf not in the light again dropped, as was expected.

Another experiment was carried out in the controlled environment chamber at Berkeley, which Professor A. R. DAVIS placed at the writer's disposal. The chambers were so constructed that the air temperature, humidity, air flow, and light intensity were under accurate control. In this case a potted strawberry plant, and rooted leaves of citrus growing in a

nutrient solution in quart jars were used. One thermocouple (no. 1) was placed in the dark where the air was entering the chamber. Another thermocouple (no. 2) was placed at the level of the leaves but exposed to direct light and not shielded from the cold box. Thermocouples (nos. 3, 4) were placed in strawberry and citrus leaves. Other thermocouples were placed in other leaves but the readings ran parallel to those here presented so they are omitted. Until near the end of the experiment, the relative humidity was kept constant at 36 per cent. and the air flow was constant at about 30 feet per minute near the leaves. In this experiment the light did not come through the tube in the cold box, but ten 500-watt lamps were shining on the plants from outside of the glass walls of the control chamber. The cold box with the insulating shield over its face was placed 27 cm. from the leaves and thermocouples. Only thermocouple no. 1 was below and completely shielded from the cold box. Readings were taken every 5 minutes. The data are presented in figure 3.

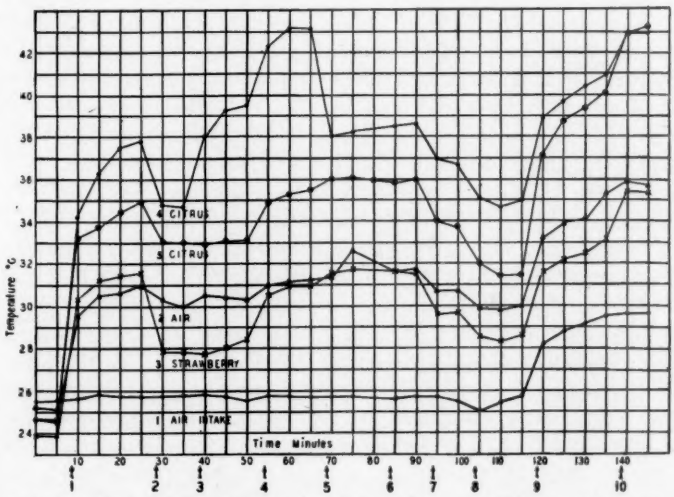


FIG. 3. Cooling of leaves by radiation to a cold box. First two readings all thermocouples in the dark. Nov. 30, 1934. At point *1*, lights were turned on; *2*, shield removed from cold box; *3*, cellophane envelope slipped over citrus leaf no. 4; *4*, shield replaced on cold box; *5*, cellophane envelope removed from citrus leaf and placed on thermocouple in the air; *6*, cellophane removed from no. 2 and placed behind no. 4 which accounts for its slow rise; *7*, shield removed from cold box; *8*, rate of air flow considerably increased; *9*, air flow completely stopped; *10*, shield replaced on cold box.

The first few readings were taken in darkness. At point *1* the lights were turned on, and the temperature of the leaves rose abruptly. At point *2* the

shield was removed from the cold box and the temperature of the leaves dropped abruptly although the air temperature, even that of the freely exposed thermocouple, changed but slightly. At point *3* a cellophane envelope was placed over the citrus leaf (no. 4) causing abrupt rise in leaf temperature. At point *4* the shield was placed over the cold box. At point *5* the cellophane envelope was removed from the citrus leaf and placed over the bare thermocouple. At point *6* the cellophane was removed from the bare thermocouple (no. 2) and placed behind leaf no. 4, which accounts for its slight rise. At point *7* the shield was again removed from the cold box. At point *8* the rate of air flow was considerably increased but the amount of increase was not measured. At point *9* the air flow was completely stopped and the temperature in all cases rose abruptly. At point *10* the shield was replaced on the cold box. From these experiments it is obvious that the temperature of objects at a distance may markedly affect the temperature of the leaves, and this effect is independent of the air temperature in the vicinity of the leaf.

In order to test the effect of radiation to the sky on the temperature of leaves, thermocouples were threaded into leaves of an orange tree at Riverside, California.[2] This experiment is somewhat complicated by the fact that the leaves had previously been whitewashed with lime to determine the effect of this treatment on leaf temperature; but similar behavior was obtained with unsprayed leaves, and since this same tree was used on a cloudy day for comparison these data rather than others are here presented. The experiment was performed on the afternoon of January 3, 1935. There were a few light clouds in the sky and by the time this particular set of readings began, at 3:40 P.M., the sun was low in the west and soon was hidden behind the clouds. Thermocouples were inserted into three leaves on the northeast side of the tree where they were exposed to the sky but shielded from the sun by the remainder of the tree. For the most part, the air movement was not strong. The thermocouple for reading air temperature was hung fairly close to the leaves. Three others, hung in various parts of the tree, when read at various intervals were found to read about the same as this one but were not read so regularly. The temperature readings were made mostly at 1-minute intervals. The data are presented in the curves in the upper part of figure 4.

At the beginning it is apparent that the three leaves were cooler than the air by about one degree. At point *1* a cardboard shield about 40 cm. wide × 50 cm. long was held at a distance of about 50 cm. from the leaves to shield them from the sky. The leaf temperatures immediately rose to approximately that of the air. At point *2* the shield was removed and the

[2] The writer is indebted to Professors H. S. REED and E. T. BARTHOLOMEW for supplying facilities for this work at the Citrus Experiment Station.

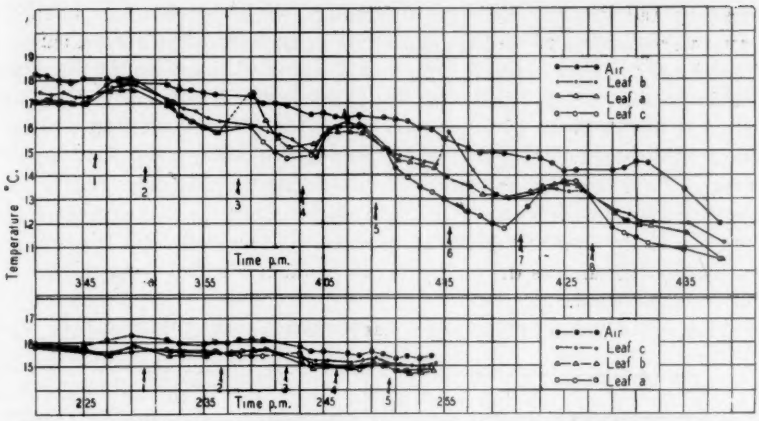


FIG. 4. Radiation to sky from citrus leaves on clear and cloudy days compared. Upper curves, data on a clear day: Up to point *1*, leaves freely exposed to the sky and cooler than the air. Points *1*, *4*, *7*, leaves shielded from sky; *2*, *5*, *8*, shield removed; *3*, lime wiped from leaf *a*; *6*, lime wiped from leaf *b*. Lower curves, data on a cloudy day: Points *1*, *4*, leaves shielded from the sky; *2*, *5*, shield removed; *3*, lime wiped from leaf *a*.

leaf temperatures immediately dropped below that of the air. At point *3* the lime was wiped from leaf *a*. Leaf *c* was close to leaf *a* and the handling of *a* and partial shading of *c* accounts for the brief rise in temperature. At point *4* the leaves and free thermocouple were again shielded from the sky, and this resulted in a rise in leaf temperature. At point *5* the shield was again removed. At point *6* leaf *b* was wiped free from the whitewash. At point *7* the leaves were shielded from the sky and there was a rise in leaf temperature. At point *8* the shield was again removed and this was followed by the expected fall in leaf temperature. The shield was again replaced and again removed with response similar to those just recorded.

The next day, January 4, the sky was completely overcast with clouds and the experiment was repeated, using similar leaves from the same side of the tree. The temperature readings are given on the curves in the lower part of figure 4. At the beginning the leaves were open to the sky. At points *1* and *4* the leaves were shielded from the sky as on the previous day, and at points *2* and *5* the shield was removed. At point *3* leaf *a* was wiped free from lime. It is clear that on this cloudy day the presence or absence of the cardboard shielding or not shielding the leaves from the sky had almost no effect upon the leaf temperatures.

It seems that on the cloudy day there was little cooling by radiation to a cold sky, while on a clear day the leaf temperatures were greatly influ-

enced by radiation to the sky. A similar fall in temperature when the leaves were exposed to the sky, and a rise to air temperature when shielded from the sky were observed for leaves of *Quercus wislizenii* on the top of Mt. Diablo near Berkeley, California, on October 19. Similar data were also obtained for leaves of *Chenopodium* sp. on top of Mt. Rubidoux at Riverside, California. At this time there was such a heavy wind blowing that it was difficult to hold the paper for recording the readings, and to keep the thermocouples in place. In spite of this wind the leaves were cooler than the air by approximately 2° C. when not shielded from the sky, and practically the same as the air temperature when shielded from the sky but not from the wind.

That air movement may have a marked effect on leaf temperature, when

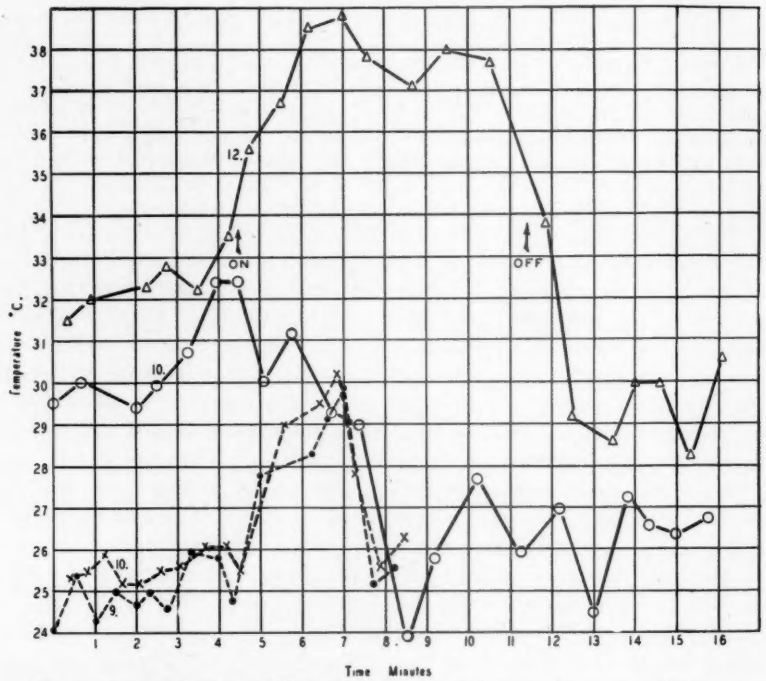


FIG. 5. Curves showing rapidity of change in leaf temperature when light is constant and air currents vary. Berkeley, Calif., Oct. 10, 1934. Broken lines: two thermocouples threaded into the same leaf. Solid lines: no. 10, indicates natural fluctuations owing to changes in air current; no. 12, leaf inclosed in cellophane envelope at "on," envelope removed at "off." Note marked rise in temperature and relatively slight drop coinciding with minimum temperature of freely exposed leaf.

the leaves are in direct sunlight and therefore much warmer than the air, has been noted by a number of investigators, notably, SMITH (**12**) and CLUM (**4**). Additional data are here given to show the rapidity of the fluctuation over time intervals that are, for the most part, shorter than those previously reported.

The data for figure 5 were obtained from leaves of *Pitosporum undulatum* on the campus of the University of California at Berkeley. The broken lines give the readings of two thermocouples threaded into the same leaf. Thermocouple no. 9 was so threaded that the tip of the thermocouple lay on the upper surface of the leaf, while thermocouple no. 10 was so threaded that the tip lay on the under surface. The readings were made first with one thermocouple and then with the other in rapid succession. The time of each reading was recorded by a stop-watch. Air temperature readings were made just before and at the end of this run and were 20.0° and 19.7° C.

The solid lines of figure 5 represent readings taken from this same leaf (to which thermocouples nos. 9 and 10 were attached) and also from a second leaf (no. 12) over which a cellophane envelope was placed and removed at the times indicated by arrows. These readings were made shortly following those just described, and the air temperatures, at the beginning and end, were 19.7° and 19.9° C.

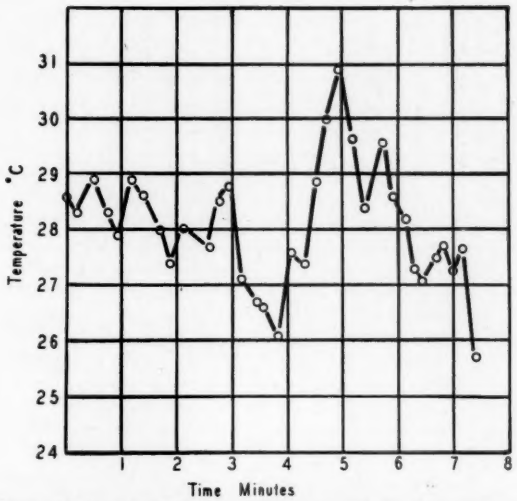


FIG. 6. Curve showing rapidity of natural changes in temperature of a leaf due to varying air currents. Air temperature immediately preceding these readings was 20.5° C., immediately afterwards, 19.7° C.

The readings for the curve in figure 6 were made immediately before those for thermocouples nos. 9 and 10 of figure 5. In this case, the temperature changes taking place in a single leaf were followed as rapidily as possible. On the average, 4.3 readings per minute were made over a period of 7.5 minutes. At the beginning the air temperature was 20.5° C., at the end 19.7° C.

The leaves were in direct sunlight for these three sets of curves and the light intensity seemed constant. With the exception of the leaf temporarily inclosed in a cellophane envelope, the temperature fluctuations seemed entirely due to fluctuations in rates of air movement. Since the leaves were so much warmer than the air it is obvious that changes in rate of air movement would greatly affect their temperature. No attempt was made to keep a careful record of air movement but it was noted in all three sets of curves that the air was quiet at the points of maximum temperature and a breeze was evident at the minimum points. It is to be noted that leaf no. 12 when inclosed in a cellophane envelope showed only a slight drop in temperature when its mate showed the minimum. This time coincided with the maximum breeze.

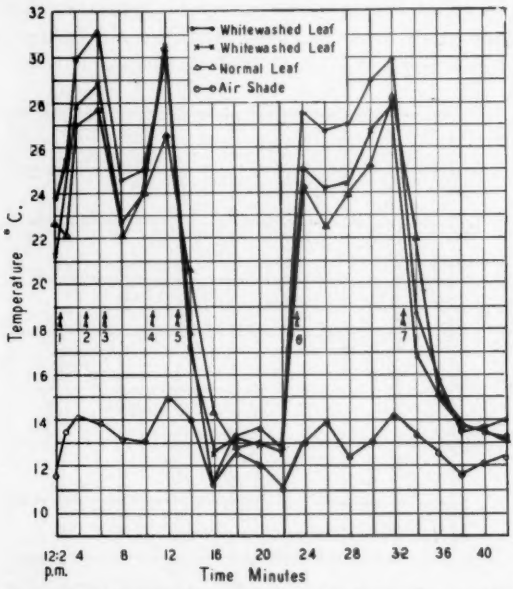


FIG. 7. Temperature changes in citrus leaves, outdoors, Jan. 6, 12: 2 to 12: 44 P.M. At point *1*, a slight breeze was noticeable; *2*, it was quiet; *3*, a breeze was again noticeable; *4*, more quiet; *5*, leaves were shaded from sun by sheet of cardboard; *6*, shade was removed; *7*, leaves were again shaded. At 12: 48, shade was removed but all temperatures fluctuated between 11° and 15° as a cloud shut off direct sunlight.

The data for figure 7 were taken on Jan. 6, 1935, from orange leaves at Riverside. In this experiment the temperatures of six thermocouples were recorded every 2 minutes, and part of the time temperatures of eight thermocouples were recorded. Curves for only 4 of these thermocouples are presented in the figure. The rapidity and degree of temperature change, changes in rate of air movement, and the effect of shading are particularly striking.

The writer and his students have frequently observed that, when measuring in a potometer the transpiration of leafy shoots exposed to strong artificial light, the transpiration is often reduced when the shoot is vigorously fanned. This, to the uninitiated, is contrary to expectation because one expects the rapid air movement to increase transpiration. When one realizes, however, that the fanning may cause a drop in leaf temperature of from a few to 10° C. or more, the marked drop in vapor pressure of the leaf owing to its fall in temperature can easily be seen to account for the decreased transpiration, and the divergent responses of leaves and evaporimeters to air currents.

In the voluminous literature dealing with various factors influencing transpiration, it seems that the importance of the effect of leaf temperature upon the process has been too little recognized. A failure to recognize that the leaf temperatures may be far different from the air temperature and that they may fluctuate over short time intervals, as indicated in figures 5, 6, and 7, has led in many cases to misinterpretation of data, particularly in relation to the effects of stomatal movement, relative humidity, and air movements. A change of only 1° to 2° C. in leaf temperature, for example, may bring about a change in vapor pressure gradient between the leaf and the atmosphere equivalent to changing the external humidity as much as 5 to 14 per cent. Assuming that at the beginning the leaf has the same temperature as the air and that the intercellular spaces remain saturated,[3] a rise in temperature of the leaf to 1° C. above the air temperature would be comparable, in its effect on transpiration, to lowering the external humidity by 5.5 to 6.9 per cent. over the temperature range between 40° and 10° C. On the same assumption, a rise of leaf temperature of 5° C. would have an effect on the vapor pressure gradient comparable to lowering the external humidity by 30.1 to 38.7 per cent., and a rise of 10° C. to a lowering of humidity by 65.5 to 90.0 per cent. For example, raising the leaf temperature from 10° to 11° C. would be

[3] With the large exposure of cell surfaces within the leaf and the short distances involved, the relative humidity is likely to remain close to 100 per cent., and in the deeper tissues probably rarely drops below 95 per cent. because the turgor deficit (suction tension) in equilibrium with 95 per cent. relative humidity would be of the order of that of a solution with an osmotic concentration equivalent to seventy atmospheres, and leaves rarely approach this concentration.

equivalent, in its effect on the vapor pressure gradient between leaf and air, to lowering the external humidity by 6.9 per cent. (that is from 100 to 93.1 per cent. or 60 to 53.1 per cent., etc.); while raising the leaf temperature from 40° to 41° C. would be equivalent to lowering the external humidity 5.5 per cent. Within the range of temperature between 10° and 40° C. if the leaf has the same temperature as the air and the air humidity is 65 per cent., transpiration would increase more by raising the leaf temperature 10° C. than would result from lowering the air humidity to 0 per cent., because raising the leaf temperature 10° C. would be equivalent to lowering the external humidity 65.5 to 90 per cent., depending on whether the original temperature was 40° or 10° C.

One of the commonest mistakes found in the literature and texts dealing with transpiration is the claim that a rise in air temperature increases transpiration because it lowers the relative humidity of the atmosphere, or increases its vapor pressure deficit. The change in relative humidity or vapor pressure deficit of the atmosphere around the leaf, however, when it is brought about by a rise in temperature of the atmosphere, does not lower the vapor pressure of the atmosphere and has no tendency whatever to increase transpiration unless the leaf also is heated, and this heating of the leaf alone is responsible for the increased transpiration. In other words, lowering the relative humidity of the atmosphere or increasing its vapor pressure deficit by raising its temperature is not responsible for increased transpiration.

In experiments performed by the writer, only a few of which are here reported, he has in no case found leaf temperatures in direct sunlight to be below that of the air surrounding them. MILLER and SAUNDERS (**9**), and EATON and BELDEN (**7**), however, have reported leaf temperatures in strong direct sunlight to be close to, and in many cases even below that of the surrounding air. It has been suggested that these low temperatures, especially those of EATON and BELDEN, are due to the fact that the experiments were carried out in such an arid environment that transpiration was sufficiently rapid to cause the marked cooling of the leaf below air temperature. When transpiration was greatly reduced by withholding water, the leaf temperatures rose only about 2 to 5° C., or approximately the same amounts observed by CLUM (**4**), and SMITH (**12**), who worked under more humid conditions and who found leaf temperatures in direct sunlight often 10° to 15° C. above air temperature. As reported in an earlier paper (**6**), it seemed possible that the lower temperatures reported by MILLER and SAUNDERS (**9**), and EATON and BELDEN (**7**) might be largely due to greater loss by radiation in the drier regions in which these investigators worked. Although the evidence presented in the present paper indicates that this loss by radiation to a clear sky, especially when the

amount of water vapor in the air is low, may be appreciable, the amount of such cooling by radiation even with increased cooling by greater transpiration, probably does not fully account for the low leaf temperatures reported by these investigators. The writer has obtained rather clear-cut evidence that particularly the methods used for determining the air temperatures may have been faulty.

MILLER and SAUNDERS (9) measured leaf temperatures by mounting one junction of the thermocouple on cork tipped clamps with which the leaf was grasped. Using a similar clamp, the writer has made many readings, comparing them with those of a thermocouple threaded into a leaf. The experimental procedure was first to read the thermocouple threaded into the leaf, then the one attached to the clamp and held in the air but shaded from the light, then this same couple held on the surface of the leaf. The three readings could be made in about 30 seconds or less. Data from such experiments in artificial light are presented in table I. The leaf

TABLE I

COMPARATIVE READINGS OF LEAF TEMPERATURES OBTAINED WITH THERMOCOUPLES EITHER THREADED INTO LEAF, OR PLACED ON LEAF SURFACE ACCORDING TO METHOD OF MILLER AND SAUNDERS

| LEAVES AND LIGHT INTENSITY | AV. NO. READINGS | TEMPERATURE | | | | |
|---|---|---|---|---|---|---|
| | | THERMOCOUPLE IN LEAF | CLAMP IN AIR | CLAMP ON LEAF | AIR SHADE | LEAF MINUS CLAMP ON LEAF |
| | | °C. | °C. | °C. | °C. | °C. |
| *Bryophyllum* in strong light | 20 | 38.3 | 29.0 | 35.3 | 25.6 | 3.0 |
| Apple in strong light | 18 | 32.5 | 27.9 | 28.8 | 25.9 | 3.7 |
| *Bryophyllum* in diffuse light | 5 | 22.6 | 23.8 | 23.5 | 23.2 | −0.9 |
| Apple in diffuse light | 2 | 21.4 | 23.8 | 23.0 | 23.2 | −1.6 |

temperature readings with the cork tipped clamp were always[4] intermediate between the temperature of the clamp when held in the air and that of the thermocouple inserted in the leaf. Therefore when the leaves were warmer than the air, as in strong sun or artificial light, the readings with the clamp were too low, while when the leaves were in diffuse light and cooler than the clamp in air readings, the readings with the clamp on the surface of the

[4] If the clamp was so held, however, that the cork face of the jaw opposite the thermocouple was held in direct light, then, especially with thin leaves, when a leaf was grasped with the clamps the temperature was higher than either the leaf or the thermocouple because the cork was heated.

leaf were too high. As was expected, the leaf readings with the clamp were more nearly correct with the thick leaves of *Bryophyllum* than with the thin leaves of apple where the heat retaining capacity of the leaves is less (see last column in the table).

In several instances MILLER and SAUNDERS (9) report leaf temperatures in direct sunlight as lower than the air temperatures. The writer has yet to find in his experiments such readings under natural conditions. In comparing the readings of a bare thermocouple hanging free in the shade with those of a thermocouple in the cork tipped clamps when so placed that the thermocouple was shaded by the cork, the writer found that the thermocouple touching the cork, although shaded, was consistently warmer than that hanging free. For example, in a series of fourteen readings each, a thermocouple in the cork jaws of the clamp but shaded from direct light by the cork showed an average temperature of 30.7° C. This was the method used by MILLER and SAUNDERS in obtaining air temperatures. A second thermocouple, about 2 cm. behind and in the shade of a vertical wooden pot label, showed an average temperature of 28.0° C. A third thermocouple in direct light read 29.9° C. A fourth thermocouple, 2 cm. behind and in the shade of a vertical apple leaf read 28.5° C. A fifth thermocouple threaded into the leaf read 33.0° C. When left in the same position but in the breeze of an electric fan, averages of 5 readings each gave the following temperature readings: 26.1°, 25.4°, 26.7°, 25.8° and 27.3° C. From these data as well as those of table I it is evident that the thermocouple shaded by the cork but in contact with it was usually 2 to 3° C. warmer than one held in the shade at a distance of one or two centimeters from the shading object. It seems from this that the air temperature readings of MILLER and SAUNDERS were too high and therefore that the leaves in direct sunlight were probably not cooler than the air as their data would indicate.

EATON and BELDEN (7) did not measure actual leaf temperature but attempted to compare leaf temperature with that of the air by placing one junction of the thermocouple in contact with the leaf and leaving the other in the air. The direction and amount of swing of the galvanometer were taken as measures of the direction and amount of temperature difference between the leaf and the air. In making the readings they placed one junction on the surface of the leaf and quickly folded the two halves of the leaf against the junction by the use of cork tipped clamps. This should give a fairly accurate measure of the leaf temperature because the thermocouple was in contact only with the leaf surfaces. It would seem a desirable method for measuring temperatures of large numbers of leaves but is valueless for following temperature changes of any given leaf with natural

or artificial environmental changes, and, furthermore, the effect of brief shading has not been determined.

What seems to be the most serious weakness in the method, as EATON and BELDEN used it, is that, to get the air temperature, this junction was "protected from the direct rays of the sun by two slightly separated slips of white paper." Their description of this paper arrangement is not sufficiently clear to enable one to follow it exactly. The writer has attempted to test the effect of a similar arrangement based on their description, including figure 2 of their article. In an experiment performed indoors using ordinary mercury thermometers, placing one between two slips of paper, and comparing the temperature of this thermometer with that of another in direct sunlight, using a third thermometer with a blackened bulb (blackened by dipping in shellac and then lamp black), and a fourth thermometer shielded by a vertical white card placed 5 cm. from the bulb, the writer found readings of the sort given in table II. In this experiment

TABLE II

COMPARISON OF TEMPERATURE READINGS WHEN MERCURY THERMOMETERS ARE VARIOUSLY SHADED FROM SUNLIGHT, INDOORS. THERMOMETERS MOSTLY IN A ROOM WITH SUNLIGHT COMING THROUGH A WINDOW. ALL READINGS BETWEEN 4 AND 4:30 P.M. DURING MAY. AVERAGES OF 2 TO 7 READINGS ARE GIVEN. VERTICAL COLUMNS NUMBERED 1 TO 4 REPRESENT DIFFERENT DAYS

| THERMOMETER IN RELATION TO SUNLIGHT | TEMPERATURE | | | |
|---|---|---|---|---|
| | *1* | *2* | *3** | *4* |
| | °C. | °C. | °C. | °C. |
| Thermometer shielded from direct sunlight by vertical white card at distance of 5 cm. | 27.1 | 26.0 | ..... | 24.8 |
| Bare thermometer in direct sunlight | 32.4 | 28.5 | 13.1 | 27.0 |
| Thermometer shielded by horizontal strips of white paper† | 37.5 | 32.5 | 16.0 | 30.4 |
| Excess over that in direct sunlight | 5.1 | 4.0 | 2.9 | 3.4 |
| Excess over air | 10.4 | 6.5 | ..... | 5.6 |
| Thermometer with blackened bulb | 43.0 | 37.0 | ..... | 36.4 |

* Placed on window ledge outside, where there was a strong wind.

† Method used was similar to that of EATON and BELDEN for obtaining air temperature.

the use of white paper strips, close to but not touching the thermometer, resulted in temperature readings averaging 2.9° to 5.1° C. higher than those of the thermometer directly exposed to the sun, and 5.6° to 10.4° C. higher than the truer air temperatures.

Similar experiments were performed with thermocouples out-of-doors.

The data of a typical set-up are summarized in table III. Readings were taken every two or three minutes. The temperatures showed many changes

TABLE III

COMPARISON OF TEMPERATURES OF THERMOCOUPLES WHEN VARIOUSLY SHADED FROM DIRECT SUNLIGHT, OUT-OF-DOORS. TEMPERATURE READINGS FROM 3:43 TO 5:17 P.M. JULY 17, 1935. AVERAGES OF 25 CONSECUTIVE READINGS ARE GIVEN

| NUMBER | THERMOCOUPLE | TEMPERATURE | | | | |
|---|---|---|---|---|---|---|
| | RELATION TO SUNLIGHT | MAX. | MIN. | AV. | AV. EXCESS ABOVE AIR | MAX. EXCESS ABOVE AIR |
| | | °C. | °C. | °C. | °C. | °C. |
| 1 | Thermocouple shielded by vertical wooden pot label 2 cm. wide at a distance of 2 cm. Assumed to be air temperature | 31.4 | 26.9 | 29.6 | 0.0 | 0.0 |
| 2 | Shielded by vertical strip of white paper at a distance of 2 cm. | 32.4 | 29.5 | 31.3 | 1.7 | 4.4 |
| 3 | Bare thermocouple (near no. 4) | 34.7 | 30.8 | 32.5 | 2.9 | 5.6 |
| 4* | Thermocouple shielded by horizontal strips of white paper | 41.7 | 35.3 | 37.7 | 8.1 | 12.2 |
| 5 | Bare thermocouple (near no. 6) | 34.9 | 27.7 | 31.8 | 2.2 | 4.5 |
| 6* | Thermocouple shielded by horizontal strips of white paper | 37.8 | 32.5 | 34.8 | 5.2 | 8.0 |
| 7* | Thermocouple shielded by horizontal strips of white paper | 39.4 | 33.4 | 36.6 | 7.0 | 10.7 |

* Method used similar to that of EATON and BELDEN for obtaining air temperature.

associated chiefly with changes in air currents. But in no case did the temperature curve of any given treatment cross that of a different treatment. That is, the bare thermocouples (nos. 3 and 5) were always warmer than the one shaded by a wooden stick (no. 1) and always cooler than any of those shielded on both sides by white paper (nos. 4, 6, and 7). Although the paper shields were open both at the side and the end and did not touch the thermocouples, they served to trap the heat and give excessively high readings. It seems probable that the air junctions in the experiments of EATON and BELDEN were also at temperatures higher than the air and therefore the leaves in direct sunlight were not as cool as indicated in their readings. The claim, therefore, of MILLER and SAUNDERS, and EATON and BELDEN that leaves in direct sunlight are often cooler than the air seems questionable.

## Discussion

Although those who have investigated thermal emissivity of leaves have mentioned the loss of heat by radiation as well as by conduction to the atmosphere, in those reports which the writer has read, the assumption is made that there will be no loss of heat by radiation unless the leaf is cooler than the atmosphere around it. The fact that the air is mostly transparent to infra-red radiation and that plant tissues may lose or receive heat by radiation to or from distant objects does not seem to be generally recognized by botanists. The temperature of the walls of a chamber may have a marked effect upon the temperature of leaves of a plant within that chamber which is entirely independent of the temperature of the air within the chamber. This in turn will influence the various physiological processes in the plant. Therefore for interpretation of certain physiological experiments, air temperature readings alone may be highly misleading. The cooling of plants at night below air temperature is undoubtedly chiefly due to loss by radiation. This cooling may lead to condensation of moisture on leaf surfaces and may thus modify turgor relations, water loss, and infection by bacteria and fungi. Such condensation does not often occur in greenhouses, even though the humidity is high, because the glass tends to prevent this loss of heat by preventing radiation to the sky.

Many papers have been published in which the effects upon transpiration of various spray residues and dusts have been reported. The fact that these materials often appreciably alter the temperature of the leaves, and therefore influence both transpiration and other processes including resistance to the toxic effects of the applied materials, seems often overlooked. Data on this phase are accumulating and will be published later.

Several writers have claimed that, if it were not for the cooling effect of transpiration, leaves would be quickly killed in direct sunlight because of excess heating. It is true that transpiration invariably tends to lower the temperature of the transpiring leaf but the data obtained by various investigators indicate that this lowering of temperature commonly amounts to less than 2° to 5° C. There are several other factors concerned in the prevention of excess heating. The angle of exposure to incident radiation will obviously greatly affect absorption of radiant energy, and therefore the temperature. SHULL (**10**) has demonstrated that with many plants 25 per cent. or more of the light within the visible spectrum is reflected even when the incident radiation is normal to the leaf surface. Photographs taken with radiation in the near infra-red indicate that many plants also reflect much of the radiant energy within this range of shorter infra-red, for they appear white in such pictures. When leaf temperatures exceed that of the

air there may be considerable loss by conduction (WATSON, **13**), especially when the air is in motion (BROWN and ESCOMBE, **2**). There is also loss by radiation to cooler objects near or far, or, when the sky is clear, to space or to cold gases, especially $CO_2$ and $H_2O$. Plant temperatures therefore may be greatly influenced by water in the atmosphere, either as clouds or vapor, at a great distance from the plant because of the influence of this water on the receipt or loss of infra-red radiation. This effect may be totally independent of the effect on transpiration of water vapor close to the leaf, and independent of the effects of clouds on the visible radiation reaching the plant. The relative importance of these various methods of dissipating heat varies greatly with the environment of the plant as well as the condition within the leaf, including also its surface. It is possible to control conditions so that any one of the more important factors, such as conduction, reduced absorption,[5] reradiation or transpiration may play a dominant rôle in preventing excessively high leaf temperatures. Often transpiration is assumed to be the only factor, or the major factor, in preventing excessive heating. There seems to be no justification for such an assumption. The available evidence points strongly to conduction to the atmosphere and reduced absorption as of major importance under natural conditions.

For the experiments reported in this paper one may well question what part transpiration played in determining the temperatures observed. Transpiration may have played a small part in some cases but the part was relatively so small that slight changes in air currents, angle of incidence, and light intensity undoubtedly would greatly overshadow any such effects. Furthermore, in several instances cut leaves, in which transpiration was practically zero, and dry pieces of black paper, showed responses to the various treatments exactly comparable to those of the attached leaves. It seemed therefore entirely useless to attempt to determine transpiration rates in these experiments.

## Summary

1. Leaf temperatures may be considerably influenced by exchange of infra-red radiation between the leaf and other materials near or at a distance from it. There may also be loss of heat by radiation to space. These effects are independent of the temperature of the air in the vicinity of the leaf because the oxygen and the nitrogen of the air are almost transparent to infra-red radiation.

2. It has been demonstrated, both under laboratory and field conditions,

[5] Both changes in angle of incidence and reflectivity of surface may influence absorption.

that leaf temperatures may be rapidly changed several degrees by allowing or preventing radiation to cold objects or to space.

3. Rapid and great changes in temperature of leaves in direct sunlight are also brought about by natural or artificial changes in rates of air flow or light intensity. The presence of water in the atmosphere either as vapor or clouds may influence plant temperatures through its effect on infra-red radiation.

4. Data, which have been taken by various investigators to demonstrate that leaves in direct sunlight may be cooler than the air, are questioned on the basis that the readings for air temperatures were probably too high.

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# MAINTAINED ELECTRICAL POLARITIES IN REGION OF THE AXILLARY BUDS IN *PHASEOLUS MULTIFLORUS*[1]

W. S. Rehm
(WITH FIVE FIGURES)

## Introduction

A theory of cell correlation has been formulated by Lund (3) in which it is suggested that the continuous electrical currents produced by polar cells may control and modify the magnitude and direction of various processes in other cells. Lund and his coworkers have accumulated considerable evidence showing definite correlations between maintained electrical potentials and cell processes.

One of the most interesting and familiar examples of cell correlation is the inhibition, in certain plants, of the normally dormant axillary buds and the subsequent release of this inhibition after the decapitation of the plant. At the end of a series of extensive experiments mainly on *Phaseolus multiflorus,* McCallum (4) states: "This non-development (of axillary buds) does not seem to be due to any lack of those conditions that favor growth, as nutrition and moisture, or to such influences as light and gravity, or to a lack of definite 'formative substance,' but to some influence, independent of all these, which an organ, acting perhaps along protoplasmic connections, is able to exert over other parts and so prevent their growth."

The work of Dostal (1) and Snow (6) indicated that a hormone-like substance was involved in this inhibition of bud development. In 1933 Thimann and Skoog (7) demonstrated that the growth substance applied to the cut surface of the decapitated plant reversibly inhibited the buds. Although the concentration of the growth substance necessary to cause complete inhibition was many times greater than the amount that could be isolated from the apex, it seems probable that the growth substance is in some manner linked with this inhibition.

The question arises, do bioelectric currents play a rôle in the inhibition and regeneration of the buds? If so, since the buds are inhibited continuously, one would expect to find electrical polarities of relatively constant orientation. The experimental work reported in this paper is therefore devoted to a study of the distribution of electrical polarities in such a plant. The scarlet runner bean, *Phaseolus multiflorus,* was used as experimental material. A discussion of the recent literature on the relation between the growth substance and potential differences will be given in a later paper.

[1] One of a series of papers on bud inhibition.

## Methods and apparatus

In most of the experiments potentials were measured with a Leeds and Northrop hydrogen ion potentiometer. Owing to the high resistance of the cutin, a Leeds and Northrop high sensitivity galvanometer was used. This gave a limit of accuracy of less than one millivolt. In some experiments the potentials were measured with a Compton Quadrant Electrometer.

Unless otherwise stated, the measurements were made on plants placed in a chamber under artificial continuous light. This chamber consisted of two identical compartments each equipped with a 1000-watt frosted bulb and a large glass-bottomed vessel through which flowed a constant stream of water which filtered out the heat rays. One compartment was used for the measurements and the other was used to grow plants and to adapt those grown in the greenhouse to the experimental conditions. Plants grown under these conditions had much longer internodes, thinner stems, and smaller leaves than plants grown in the greenhouse. HARVEY (**2**) has reported growing a large variety of plants, including beans, from seed to seed in artificial continuous light. However, plants grown in the above chambers remained in the vegetative state indefinitely. The temperature in the

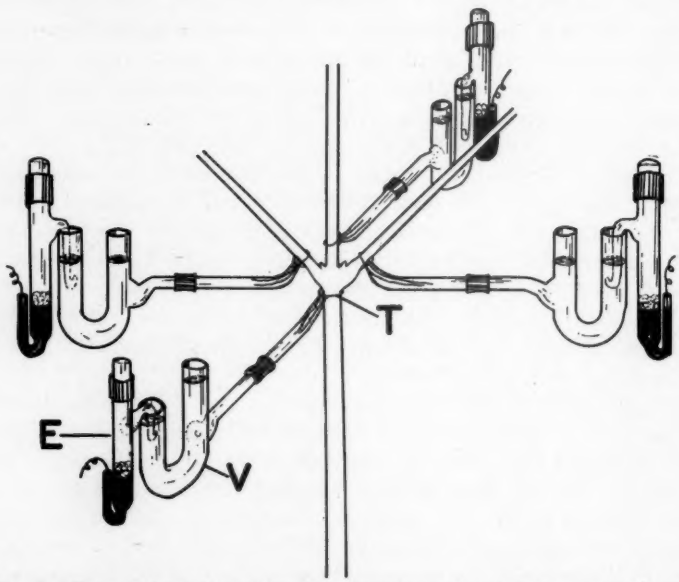


FIG. 1. Four contacts in position for measuring node potentials. Contact with the plant is made by looping a linen thread, *T*, around the plant and then placing the ends into the end of the contact cup, *V*. *E* represents a Zn—$ZnSO_4$ electrode.

chamber did not vary more than one degree over a period of hours. Plants grown in the greenhouse were placed in the experimental chamber 24 hours before an experiment.

Contact with the plant for the purpose of measuring the potentials was made as shown in figure 1. From the end of the contact cup, *V*, which contained tap water, unless otherwise specified, a linen thread, *T*, was looped around the plant. The contacts were washed by placing several drops of the same solution as that in the contact cups on the linen threads. The level of the solution in the cups was so regulated that these drops were drawn into the cups. The contact cup, *V*, was large enough to permit frequent washing of the linen thread without changing the size of the drop of solution that adhered to the plant. As many as sixteen of these contacts could be placed at one time on a given plant.

The potentials were led off from cup *V* to the potentiometer or electrometer by non-polarizable Zn-$ZnSO_4$ electrodes. The electrodes were checked before and after each series of readings. An electrode was usually placed in each vessel and, with the aid of a mercury cup switchboard that had a reversing key, as many as three readings per minute could be taken with the potentiometer.

## Orientation and behavior of maintained potential differences in *Phaseolus multiflorus*

### Preliminary experiments

In these experiments plants grown both in the greenhouse and in the experimental chamber were used. Contacts were placed along the main axis and petioles of the plants, and the potential differences were measured at intervals over a period of hours and days. When both contacts in a given measurement were washed, the potential difference would sometimes show a momentary change of about 3 or 4 millivolts, followed by a return to the original potential difference. If only one contact was washed it became more positive with respect to the unwashed contact. The magnitude of this change was usually from 4 to 6 millivolts. The potential difference would usually return to within 2 or 3 millivolts of its original value. Care was always taken to wash all contacts in the same manner. Contacts were washed before each reading except when these were taken at very short intervals. The effect of washing the contacts was the same on dead as on living plants.

In elongating internodes the orientation of potential differences was extremely variable. In internodes and petioles that had ceased elongating the potential differences, for comparable lengths, were of smaller magnitude and possessed a more variable orientation than those across the nodes and

those from the base of an internode to the base of the corresponding petioles. In figure 2 the loci of these large and relatively stable potential dif-

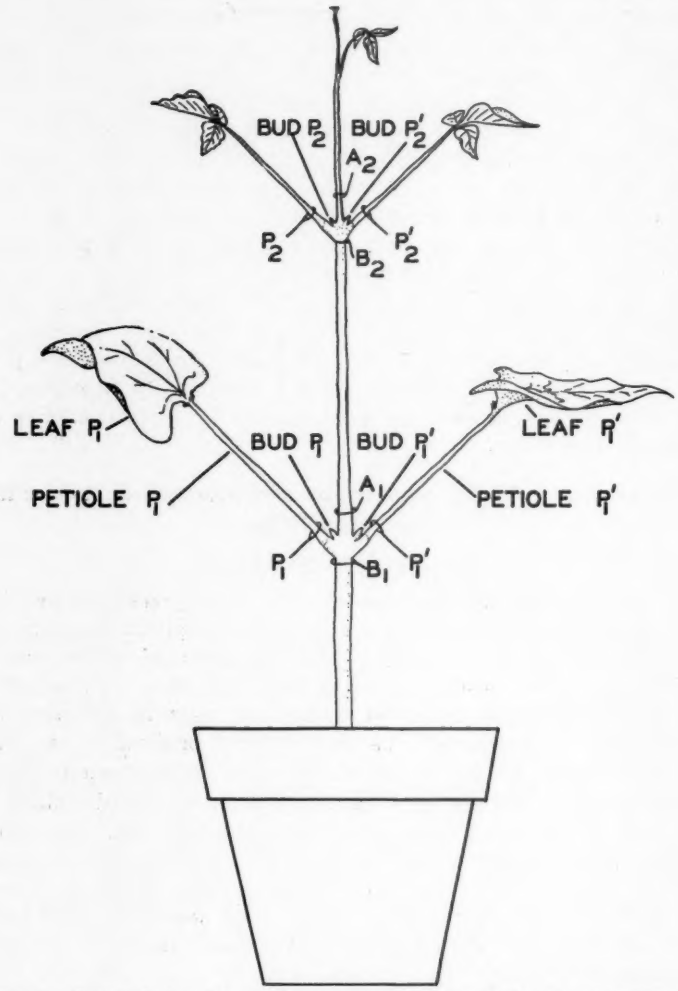


FIG. 2. Various loci and parts of the plant referred to in the text are represented in this figure. Contacts $A_1$, $P_1$, $P_1'$, $A_2$, $P_2$, $P_2'$ were all placed 1 cm. from the center of the corresponding node. $B_1$ and $B_2$ were both placed 1 mm. below the node. Potential differences between $A_1$ and $B_1$, $A_1$ and $P_1$, and $A_1$ and $P_1'$ are first node potentials; $A_2B_2$, $A_2P_2$ and $A_2P_2'$ are second node potentials.

## TABLE I

ORIENTATION OF POTENTIAL DIFFERENCES OF THE FIRST NODE. PLANTS 1, 2, AND 3 WERE GROWN AND MEASURED IN THE EXPERIMENTAL CHAMBER. PLANTS 4, 5, AND 6 WERE GROWN IN THE GREENHOUSE AND MEASURED IN THE EXPERIMENTAL CHAMBER. AT BEGINNING OF EXPERIMENT THE FIRST NODE WAS WELL DEVELOPED IN ALL PLANTS AND THE SECOND NODE WAS BEGINNING TO APPEAR.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| PLANT | LOCI MEASURED | NO. OF READINGS | NORMAL READINGS | AVERAGE P.D. | RANGE OF VARIATION OF P.D. | TEMPERATURE RANGE | DURATION OF EXPERIMENT |
| | | | % | *m.v.* | *m.v.* | °C. | *hours* |
| 1 | $A_1B_1$ | 11 | 100.0 | −22.8 | −7 to −39 | 25.5 to 29.5 | 64 |
| | $A_1P_1$ | 11 | 100.0 | −23.6 | −5 to −43 | | |
| | $A_1P_1'$ | 11 | 90.9 | −20.2 | +2½ to −39 | | |
| 2 | $A_1B_1$ | 29 | 100.0 | −23.9 | −5½ to −43 | 25 to 30 | 75 |
| | $A_1P_1$ | 29 | 100.0 | −20.6 | −6½ to −33½ | | |
| | $A_1P_1'$ | 29 | 100.0 | −25.9 | −10½ to −41 | | |
| 3 | $A_1B_1$ | 28 | 96.4 | −20.1 | 0 to −35 | 27 to 33.5 | 30 |
| | $A_1P_1$ | 28 | 100.0 | −18.3 | −3½ to −25½ | | |
| | $A_1P_1'$ | 28 | 100.0 | −16.3 | −½ to −29½ | | |
| 4 | $A_1B_1$ | 20 | 100.0 | −11.8 | −4 to −27 | 29 to 30 | 48 |
| | $A_1P_1$ | 20 | 100.0 | −17.1 | −5 to −32 | | |
| | $A_1P_1'$ | 20 | 95.0 | −17.1 | +1½ to −32 | | |
| 5 | $A_1B_1$ | 20 | 85.1 | −11.0 | +3½ to −23 | 29 to 30 | 48 |
| | $A_1P_1$ | 20 | 80.0 | −11.3 | +1½ to −26 | | |
| | $A_1P_1'$ | 20 | 70.0 | −5.0 | +15 to −20 | | |
| 6 | $A_1B_1$ | 20 | 100.0 | −29.5 | −12½ to −41 | 29 to 30 | 48 |
| | $A_1P_1$ | 20 | 100.0 | −23.1 | −10 to −39½ | | |
| | $A_1P_1'$ | 20 | 100.0 | −22.5 | −11½ to −42 | | |

ferences in the node are labelled. The potential differences from $A_1$ to $B_1$, from $A_1$ to $P_1$, and from $A_1$ to $P_1'$ will be referred to as first node potentials, and $A_2B_2$, $A_2P_2$, and $A_2P_2'$ as the second node potentials. $A_1$, $P_1$, $P_1'$, $A_2$, $P_2$ and $P_2'$ were each placed 1 cm. from a point at the center of the corresponding node. $B_1$ and $B_2$ were each placed 1 mm. below the corresponding node. The same convention with regard to the sign of the potential differences will be employed as that used in other work from this laboratory; that is, if $A_1$ is negative to $B_1$ in the external circuit, $A_1B_1$ is written with a negative sign before it; and if $A_1$ is positive to $B_1$, $A_1B_1$ is written with a positive sign before it.

### Orientation of potential differences in first and second nodes under various conditions

The orientations of the node potentials are presented by means of four tables and several figures. In tables I and II, column 3 gives the total number of readings. The readings were usually taken at 1- to 2-hour intervals during a period of 8 to 10 hours a day. Sometimes the potentials were measured at shorter intervals. The measurements taken at short intervals were grouped into periods corresponding to the time intervals between the other measurements, averaged, and the average recorded as one reading. On the plants in table III, readings were taken on the electrometer at 5-minute intervals for definite periods and the number of readings (column 3) records the actual number of measurements. Column 4 of the three tables gives the percentage of normal readings, that is, the percentage of readings in which $A_1$ or $A_2$ was negative in the external circuit to the other contact for a given measurement. In these experiments the periods of measurement were not prolonged in order to determine the duration of a given inversion. Therefore the percentage of normal readings is a meausre of the relative time the potentials possessed the normal orientation. Column 8 gives the duration of the experiment, which is the time between the first and last reading.

At the beginning of the experiments, the plants in table I had one node developed; those in tables II and III had three nodes developed. Plants 1, 2, and 3 in table I were grown and measured in the experimental chamber. Plants 4, 5, and 6 in table I and the three plants in table II were grown in the greenhouse and measured in the experimental chamber.

The three plants given in table III were grown in the greenhouse and measured in the open. These plants were protected from the wind, and this protection cut off the direct sunlight.

The plants in tables I and II were taken from a group of plants in the vegetative state. The three plants in table III were selected from a group of plants, some of which possessed elongating flower buds on the second

## TABLE II

ORIENTATIONS OF POTENTIAL DIFFERENCES OF FIRST AND SECOND NODES IN PLANTS GROWN IN THE GREENHOUSE AND MEASURED IN THE EXPERIMENTAL CHAMBER. ALL THREE PLANTS HAD THREE NODES DEVELOPED AT BEGINNING OF EXPERIMENT

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| PLANT | LOCI MEASURED | NO. OF READINGS | NORMAL READINGS | AVERAGE P.D. | RANGE OF VARIATION OF P.D. | TEMPERATURE RANGE | DURATION OF EXPERIMENT |
| | | | % | *m.v.* | *m.v.* | °*C.* | *hours* |
| 1 | $A_1B_1$ | 14 | 100.0 | −27.7 | −10 to −37½ | 24 to 28 | 60 |
| | $A_1P_1$ | 14 | 100.0 | −35.0 | −15 to −46½ | | |
| | $A_1P_1'$ | 14 | 100.0 | −40.3 | −21 to −52 | | |
| | $A_2B_2$ | 14 | 100.0 | − 9.3 | − 3½ to −14½ | | |
| | $A_2P_2$ | 14 | 100.0 | −22.0 | − 7½ to −38 | | |
| | $A_2P_2'$ | 14 | 100.0 | −22.0 | −12 to −32½ | | |
| 2 | $A_1B_1$ | 14 | 64.3 | − 9.9 | + 8 to −36 | 24 to 28 | 60 |
| | $A_1P_1$ | 14 | 85.7 | −15.2 | + 5½ to −40 | | |
| | $A_1P_1'$ | 14 | 92.8 | −14.4 | + 3½ to −32½ | | |
| | $A_2B_2$ | 14 | 100.0 | −11.9 | − 2 to −24 | | |
| | $A_2P_2$ | 14 | 92.8 | −11.7 | + 2 to −24 | | |
| | $A_2P_2'$ | 14 | 92.8 | −16.8 | +10 to −38 | | |
| 3 | $A_1B_1$ | 22 | 95.4 | − 8.9 | + ½ to −27½ | 24 to 29 | 91 |
| | $A_1P_1$ | 22 | 100.0 | −14.7 | − 7½ to −33½ | | |
| | $A_1P_1'$ | 22 | 100.0 | −13 | − 3½ to −33½ | | |
| | $A_2B_2$ | 22 | 73.7 | − 8.9 | +16 to −28 | | |
| | $A_2P_2$ | 22 | 100.0 | −34.5 | − 1 to −54 | | |
| | $A_2P_2'$ | 22 | 100.0 | −26.4 | − 2 to −53 | | |

## TABLE III

ORIENTATIONS OF POTENTIAL DIFFERENCES OF FIRST AND SECOND NODES IN PLANTS GROWN IN THE GREENHOUSE AND MEASURED IN THE OPEN. ALL THREE PLANTS HAD THREE NODES DEVELOPED AT BEGINNING OF EXPERIMENT

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| PLANT | MEASURED LOCI | NO. OF READINGS | NORMAL READINGS | AVERAGE P.D. | RANGE OF VARIATION OF P.D. | TEMPERATURE RANGE | DURATION OF EXPERIMENT |
| | | | % | *m.v.* | *m.v.* | °C. | *hours* |
| 1 | $A_1B_1$ | 132 | 100.0 | −20.1 | − 6 to −31½ | 26 to 30.5 | 46 |
| | $A_1P_1$ | 132 | 98.5 | −12.0 | + 1 to −22 | | |
| | $A_1P_1'$ | 132 | 100.0 | −17.5 | − 7½ to −24 | | |
| | $A_2B_2$ | 132 | 91.0 | − 5.3 | + 2½ to −10½ | | |
| | $A_2P_2$ | 132 | 77.2 | − 2.4 | + 5½ to − 9½ | | |
| | $A_2P_2'$ | 132 | 93.2 | −11.4 | 6 to −23 | | |
| 2 | $A_1B_1$ | 170 | 100.0 | −23.9 | − 6 to −56 | 26 to 31 | 46 |
| | $A_1P_1$ | 170 | 100.0 | −16.0 | − 4 to −49 | | |
| | $A_1P_1'$ | 170 | 93.0 | − 8.0 | +16 to −37½ | | |
| | $A_2B_2$ | 170 | 80.0 | −16.4 | + 5 to −46 | | |
| | $A_2P_2$ | 170 | 92.8 | −12.6 | +10½ to −45 | | |
| | $A_2P_2'$ | 170 | 82.3 | −13.6 | +33 to −61 | | |
| 3 | $A_1B_1$ | 147 | 74.3 | −12.0 | +10½ to −28 | 24 to 32 | 79 |
| | $A_1P_1$ | 147 | 99.4 | −14.3 | 0 to −31½ | | |
| | $A_1P_1'$ | 147 | 100.0 | −16.2 | − 4 to −28½ | | |
| | $A_2B_2$ | 147 | 100.0 | −15.7 | − 4 to −28 | | |
| | $A_2P_2$ | 147 | 70.7 | −16.8 | + 7½ to −37½ | | |
| | $A_2P_2'$ | 147 | 70.7 | −10.0 | + 7 to −26 | | |

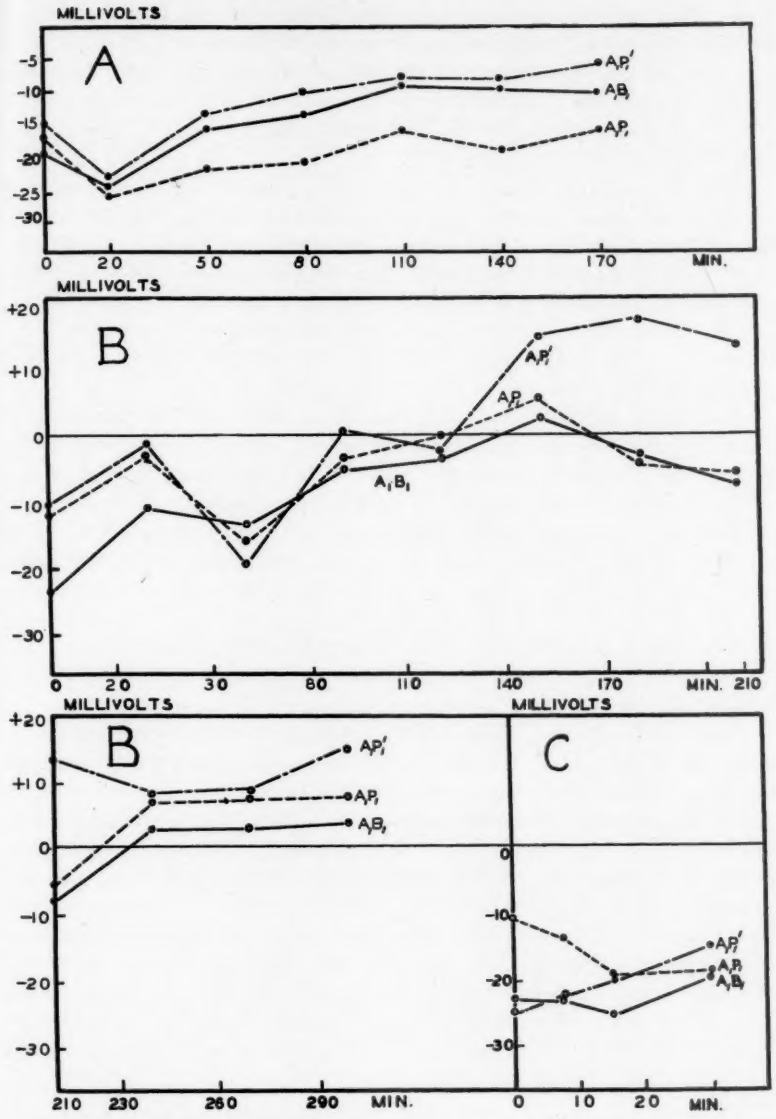


FIG. 3. Behavior of node potentials of plant no. 5 table I with respect to time on three consecutive afternoons: *A*, *B*, and *C*. Abscissas give time in minutes; ordinates give potential in millivolts.

node. The buds of the plants chosen for the experiments, however, were of average size and did not elongate during the experiments.

An examination of column 4 in tables I, II, and III shows *the node potentials under all these conditions exhibited a relatively constant orientation in which* $A_1$ *is negative in the external circuit to* $B_1$, $P_1$, *and* $P_1'$ *and that* $A_2$ *is negative to* $B_2$, $P_2$, *and* $P_2'$.

NODE POTENTIALS AND AGE OF NODE.—It may be seen from a comparison of plants 4, 5, and 6 of table I with the plants in table II that the orientation and magnitude of the first node potentials are similar. In another experiment, three plants each with two nodes developed showed a similar orientation and magnitude of the first node potentials. It may be concluded, therefore, that the orientation and magnitude of the first node potentials is maintained in plants with 1, 2, and 3 nodes developed, *i.e.*, in both young and old plants.

VARIATIONS IN THE NODE POTENTIALS.—The characteristic variations in the node potentials are illustrated in figure 3. This figure represents the variations of the node potentials of plant 5 in table I with respect to time on three consecutive afternoons. *A* of this figure, showing the behavior on the first afternoon, represents a typical case, the node potentials being characterized by comparatively slow, continuous, and spontaneous variations. *B*, showing the behavior on the second afternoon, represents an extreme case in which the variations were comparatively rapid and were accompanied by an inversion of the node potentials. An examination of table I shows that the percentage of normal readings is less for plant 5 than for the other plants in this table.

Periods of inversion occur in both nodes, as is evident from an examination of tables I, II, and III. Inversions occur without any apparent significant variations in the known external conditions. The temperature during the inversion represented in figure 3 *B* varied only one degree (from 29° to 30° C). These inversions usually last from a few minutes to several hours. Sometimes an inversion may take place in only one of the node potentials. Figure 4 *A* shows such an inversion in the node potential $A_1P_1$ that lasted only a few minutes. The inversion was preceded by a gradual decrease in the magnitude of the potential difference and followed by a gradual increase to approximately its former magnitude. Accidental movement of the plant often caused the node potentials to diminish and even to become inverted. This type of inversion, however, is followed by a rapid return to the former magnitude.[1]

[1] From the beginning of the second afternoon to the beginning of the third afternoon, represented by *C*, bud $P_1'$ grew 0.5 mm. Correlated with this is the fact that the inversion of the corresponding node potential $A_1P_1'$ was greater than that of the other node potentials. However, in following up this lead, the writer found that periods of inversion occur

### Comparison of first and second node potentials

In table IV the data of the three node potentials of a given node were averaged. Plants 1, 2, and 3 in this table are the plants in table III, and

TABLE IV

Comparison of first and second node potentials with $A_2A_1$. All readings of the three node potentials for a given node were averaged. Node potentials and $A_2A_1$ were always measured at the same reading. P.D. is recorded in millivolts

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| Plant | Readings in which $A_2$ was negative to $A_1$ | Average P.D. of $A_2A_1$ | Normal readings in first node potentials | Average P.D. of first node potentials | Normal readings in second node potentials | Average P.D. of second node potentials | Duration of experiment |
| | % | *m.v.* | % | *m.v.* | % | *m.v.* | *hours* |
| 1 ..... | 7.8 | +15.8 | 100.0 | −16.5 | 86.7 | − 6.3 | 46 |
| 2 ..... | 33.1 | + 5.3 | 98.8 | −16.0 | 86.0 | −14.2 | 46 |
| 3 ..... | 97.7 | −14.9 | 91.2 | −14.2 | 84.0 | −14.2 | 79 |
| 4 ..... | 7.1 | + 4.0 | 100.0 | −34.0 | 100.0 | −17.9 | 60 |
| 5 ..... | 35.7 | + 1.8 | 81.0 | −13.2 | 95.3 | −13.5 | 60 |
| 6 ..... | 86.4 | −14.9 | 98.5 | −12.2 | 89.4 | −16.6 | 91 |
| 7 ..... | 36.4 | + 5.3 | 100.0 | −23.7 | 99.1 | −15.1 | 23 |

were selected from a group of plants some of which possessed elongating flower buds in the second node but not in the first node. A comparison between columns 4 and 6 of this table shows that in each plant the percentage of time that the node potentials possessed the normal orientation was less for the second than for the first node. On the other hand the variation in the orientation of the node potentials in plants 4, 5, 6, and 7, all grown in the greenhouse and measured in the experimental chamber, shows no definite difference between the first and second nodes. It may be that this greater variation in the orientation of the second node potentials in plants 1, 2, and 3 may be associated with the tendency to pass from the vegetative to the flowering state.[2]

without any noticeable increase in bud length. The longest period of inversion in which the potentials were measured the entire time was 9 hours. The buds did not grow during this inversion.

[2] In only one plant were there flower buds present on the second node that definitely increased in length during the period of measurement. The potentials of the nodes were averaged in the same manner as those in table IV. The percentage of readings that possessed the normal orientation was 99.3 per cent. and 34.9 per cent. for the first and second nodes respectively. This suggests that inversion of the node potentials may be associated with the elongation of the flower buds. However, before a definite conclusion may be reached, more work must be done.

The standard deviation was calculated for plants 4, 5, 6, and 7. For the first node it was $11.95 \pm 0.55$; $10.3 \pm 0.45$ for the second node. The shapes of the frequency distribution curves were neither symmetrical nor similar, so that the difference is scarcely significant.

### Comparison of a longitudinal polarity between homologous points on two adjacent nodes with the node potentials

Table IV shows a comparison of the orientation of $A_2A_1$ with that of the potentials of the first and second nodes. The node potentials and $A_2A_1$ were always measured at the same reading on the plants represented in this table. Column 2 gives the percentages of the number of readings in which $A_2$ was negative to $A_1$. Column 3 records the average potential difference of $A_2A_1$. Plants 1, 2, and 3 of table IV were taken from a group that were passing into the flowering state.

In plant 1, $A_2$ was usually positive to $A_1$; in plant 3, $A_2$ was almost always negative to $A_1$. The same variation of $A_2A_1$ from plant to plant is also found in plants 4, 5, 6, and 7, which were all grown in the greenhouse and measured in the experimental chamber. It is evident that the node potentials show a much greater constancy in orientation than $A_2A_1$, and that under the experimental conditions the plants did not exhibit a constant longitudinal polarity.

### Behavior of node potentials in the dark

The foregoing experiments demonstrate that the potentials of the node possess a relatively constant orientation in artificial light and in normal daylight. The question then arose, are the potentials of the nodes maintained in the dark?

In the experiments reported here the plants were grown in the greenhouse and measured in the open, but protected from the wind. Figure 4*A* shows the magnitudes of the first node potentials in passing from daylight to darkness. It can be seen from these curves that the relative magnitude and orientation of the node potentials are maintained for the first hour in the dark. Figure 4*B* shows the magnitudes of the second node potentials in passing from darkness to daylight. The break in these curves was caused by washing and readjusting the contacts. It is evident from the curves that in the latter part of a period of darkness the node potentials are still maintained and that there is no immediate effect on passing into daylight.

Table V also shows the behavior of the node potentials in the darkness. In plant 1 of the table V the periods in darkness were approximately two hours (8:00 to 10:00 P.M.) on consecutive nights. The periods in light were for corresponding time intervals in the afternoon. The potentials of

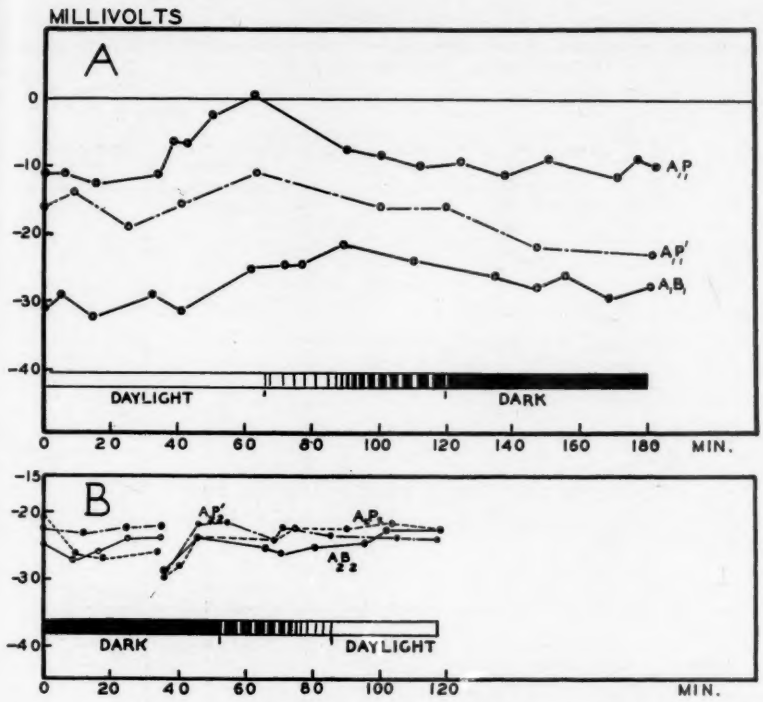


FIG. 4. *A*. Effect of passing from daylight to darkness on first node potential. *B*. Effect of passing from darkness to daylight on second node potential.

the first node were of approximately the same magnitude in daylight as in the dark. The second node was inverted during the first period in the dark. During the last period in the dark the potentials of the node were low.

In plant 2 of this table the periods of light were in the afternoon. The first period in the dark was from midnight to 3:00 A.M.; the second from 8:00 to 10:00 P.M.; and the third from 5:00 to 6:00 A.M. the next night. The node potentials were also maintained during periods of darkness in the experimental chamber. The temperature, in the experiment represented by *A* (fig. 4), varied from 28.5° to 26° C.; in that of *B* (fig. 4), it varied from 25° to 26.5° C.

It is evident from these results that the node potentials are maintained in the dark and that occasionally periods of inverted orientation may occur. The mechanism for the production of these potentials is not, therefore, directly dependent on the photosynthetic mechanism.

TABLE V

COMPARISON OF BEHAVIOR OF NODE POTENTIALS IN LIGHT AND DARKNESS. P.D. IS RECORDED IN MILLIVOLTS. FURTHER EXPLANATION IN TEXT

| Loci | Plant 1 | | Plant 2 | |
|---|---|---|---|---|
| | Average P.D.'s of three periods in darkness | Average P.D.'s of three periods in light | Average P.D.'s of three periods in darkness | Average P.D.'s of three periods in light |
| | *m.v.* | *m.v.* | *m.v.* | *m.v.* |
| $A_1B_1$ | −32.8 | −18.6 | −12.8 | −14.4 |
| | −22.5 | −21.3 | −17.4 | −10.8 |
| | −21.5 | −27.2 | −26.6 | − 5.6 |
| $A_1P_1$ | − 6.4 | −10.3 | − 6.1 | −11.4 |
| | −18.4 | −16.6 | −26.6 | −17.6 |
| | −29.0 | −15.3 | −11.7 | −12.7 |
| $A_1P_1'$ | − 7.0 | − 8.5 | − 6.1 | −11.5 |
| | −11.1 | −10.2 | −26.1 | −13.8 |
| | − 5.5 | − 6.5 | −11.7 | −15.2 |
| $A_2B_2$ | + 3.2 | −32.5 | − 7.2 | −15.8 |
| | −11.5 | −15.8 | −23.4 | −10.2 |
| | − 0.3 | −31.3 | −24.7 | −12.9 |
| $A_2P_2$ | + 1.3 | −35.5 | + 1.8 | −13.8 |
| | −12.6 | −10.3 | −34.0 | −14.2 |
| | − 4.7 | −14.4 | −23.4 | −17.1 |
| $A_2P_2'$ | +26.9 | −42.8 | − 0.6 | − 5.5 |
| | −10.3 | −24.3 | −30.1 | − 0.5 |
| | − 5.1 | −26.4 | −23.4 | − 9.7 |

MEASURED POTENTIAL DIFFERENCES ARISE WITHIN THE PLANT

Since the same solution was always used in all the contacts for any given reading, and since these contacts were frequently washed at the junction of the linen thread with the plant, it seems improbable that the potential difference measured could originate from a difference in potential at these two junctions. It may be, however, that the surface of one locus was different from that at another, and that these surfaces may give rise to different potentials even when in contact with the same solution. It is well known that the magnitudes of the various types of potentials that may originate at a boundary such as that between the solution and the plant are dependent on the ionic species of the solution. See, for example, MICHAELIS (5). Experiments were therefore performed to see if the orientation or magnitude of the potential difference was a function of the ionic species in the contact solution.

Figure 5*A* shows the behavior of $A_1P_1$ when Shive's solution and tap water were used alternately as contact solutions. In changing from one

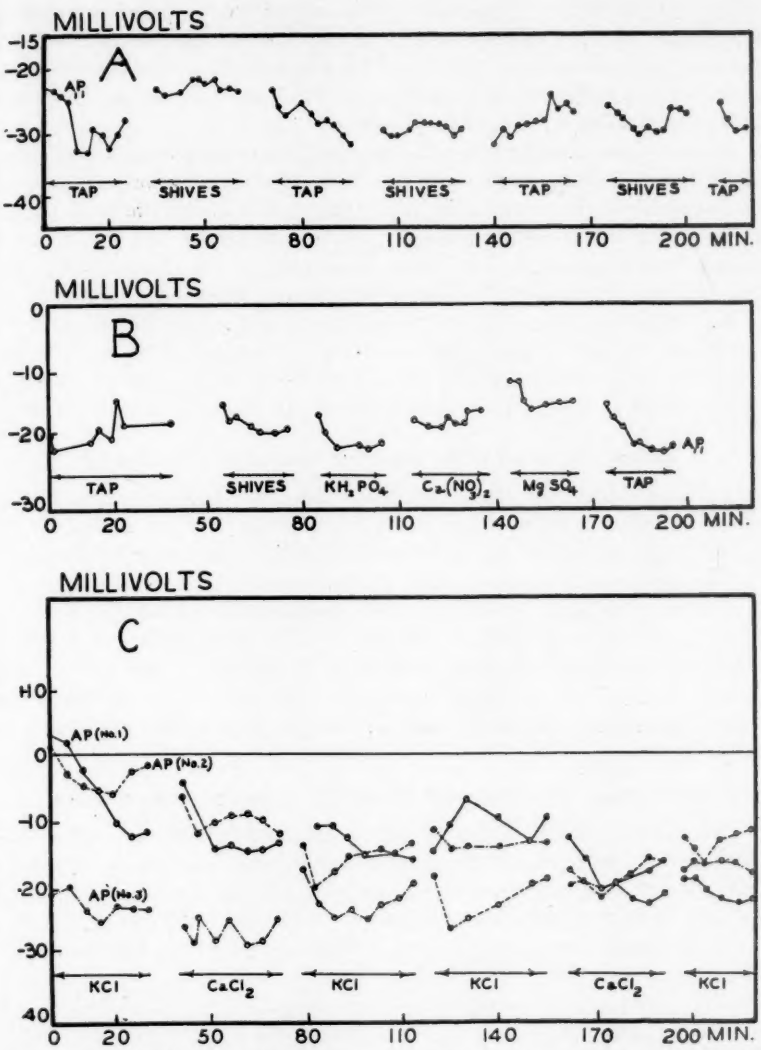


FIG. 5. *A*. Behavior of $A_1P_1$ when Shive's solution and tap water were used alternately as contact solutions. *B*. Behavior of $A_1P_1$ when various solutions were used as contact solutions. $KH_2PO_4$, $Ca(NO_3)_2$ and $MgSO_4$ solutions were 0.04 molar. *C*. Behavior of $A_1P_1$ of three plants when KCl and $CaCl_2$ solutions were used alternately as contact solutions.

solution to the other, the contact vessels were always cleaned and refilled three times with the new solution. Figure 5*B* shows the result of application of the constituent electrolytes of Shive's solution on the potential difference, *i.e.*, $KH_2PO_4$, $MgSO_4$, and $Ca(NO_3)_2$. Figure 5*C* shows the effect of $CaCl_2$ and KCl on $A_1P_1$ in three plants.

It is obvious from these experiments that the orientation is independent of the type of solution at the contacts. It can be seen also that the variation in magnitude from one solution to another is within the limits of normal variations of the potential difference. It is well known that the effects of calcium and potassium ions on living protoplasm are usually quite different. The absence of a definite difference in the experiment represented by 5*C* indicates that the solution is not directly affecting any vital processes within the tissue. It may be concluded from these experiments that in all probability very little of the measured potential differences is due to differences in the potentials at the contacts, and must therefore originate within the plant.

### Effect of killing the plant on potential differences

In three plants the first node potentials were measured immediately before and after a jet of steam was directed at the first node. Killing the node in this way caused the node to shrink considerably and the potentials to drop immediately to zero. Two plants were killed by placing them in a solution of formalin (8 per cent.) and allowing them to remain overnight. The next morning the plants were found to be quite rigid and the node potentials were of the order of magnitude of plus five to minus five millivolts. However, the large spontaneous fluctuations so characteristic of the potentials of living plants were absent, the potential differences gradually decreasing toward zero.

### Production of continuous currents by the node potentials

In order to find out whether or not the potential differences could deliver continuous currents, the following experiments were performed. Contacts containing Shive's solution were placed at $A_1$ and $B_1$ on several plants and the current was led off to a microammeter. On closing the circuit, a deflection of about 0.1 microampere resulted which was maintained as long as the circuit was closed. The microammeter was not sensitive enough, so a galvanometer, calibrated to measure currents, was used in the next experiment. In order to reduce the resistance of the circuit, the contact at $A_1$ was moved downward several millimeters. The distance between this contact and $B_1$ was now 8 mm. The circuit was closed for varying lengths of time. The circuit was broken for a minute or so when the potential differences were measured with these contacts. The potential difference between these two contacts was –10 millivolts and the current was 0.128 microampere immedi-

ately before the circuit was closed. At the end of about twelve hours the current was 0.25 microampere and the potential difference was – 29.5 millivolts. This demonstrates that the potential difference can give rise to continuous currents of this order of magnitude without a steady decrease in these potentials. Breaking the circuit for varying short periods of time did not change the deflection of the galvanometer when the circuit was remade, which indicates that the passage of this current did not appreciably polarize any part of the circuit.

## Discussion

The main purpose of this investigation was to lay the foundation for an ultimate answer to the question, Are there electrical polarities in *Phaseolus* which act as correlating agents in the inhibition of the axillary buds? The foregoing experiments have demonstrated that in *Phaseolus* there are electrical polarities in the region of the buds that possess a relatively constant orientation even under extreme environmental conditions. In sharp contrast to the relatively constant orientation of the node potentials is the variable orientation of the longitudinal polarity $A_2A_1$. This unexpected variability of $A_2A_1$ may be due to the conditions of the plants in the experiments, the plants being intermediate between the vegetative and the flowering stage.

Experiments on the effect of decapitation on the node potentials, the relation between the node potentials and the regeneration of the buds, and the effect of applied current on the node potentials and on the regeneration of the buds are well under way and will be reported in a subsequent paper. A discussion of the possible rôle of these currents and their relation to the growth substance will be given at that time.

## Summary

1. In the Scarlet Runner bean, *Phaseolus multiflorus*, certain electrical polarities in the region of the axillary buds, designated in this investigation as the node potentials, possessed characteristics that suggest the possibility of their playing a rôle in bud inhibition. Characteristics of node potentials are as follows:

(*a*). They possess a relatively constant orientation in both young and old plants.

(*b*). This relatively constant orientation is maintained in artificial continuous light, normal daylight, and darkness.

(*c*). The orientation of these potentials was also found to be much more constant than a polarity along the main axis between the homologous parts of two adjacent nodes.

2. The normal orientation of the first and second node potentials is such that for the first node $A_1$ is negative in the external circuit to $B_1$, $P_1$, and $P_1'$, and for the second node $A_2$ is negative to $B_2$, $P_2$, and $P_2'$. Occasionally periods of inverted orientation occur in the nodes without any significant changes in the known external conditions.

3. Evidence is presented to show that these measured potential differences are not artifacts due to the method of measurement, but arise within the plant; are dependent on the activities of the living cells; and can give rise to continuous electrical currents.

The writer wishes to express his gratitude to Professor E. J. LUND for valuable suggestions and criticisms. The work reported in this paper is the first part of an investigation made during the tenure of a research fellowship granted by the Department of Zoology, University of Texas.

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# GROWTH AND WATER RELATIONS OF THE AVOCADO FRUIT

A. R. C. HAAS

(WITH THIRTEEN FIGURES)

## Introduction

Some of the heaviest losses of thin skinned avocado fruit are due to end-spots, a name applied by COIT (1) to the type of spoilage that affects mature fruits on the tree. In end-spotted fruit the larger end gradually withers and dries and in some cases the cracking may proceed until the seed is exposed. In another type of end-spot called "speckles," small, dark spots occur as dry, depressed areas in the skin. Among the factors involved (6) are an overmaturity of the skin in the affected portion of the fruit and a desiccation of the fruit surfaces as a result of an extreme water deficit in the tree.

End-spots may become of greater importance in the near future because of the popularity of thin skinned fruit and the need for additional varieties with maturity dates covering the entire season.

The present paper[1] considers certain phases of the growth and water relations of the avocado fruit. Chemical studies to be reported later furnish data that may aid in gaining an understanding of these physiological diseases. No conclusions are drawn regarding the horticultural application of the results obtained.

## Investigation

### GROWTH OF FRUITS

From a practical standpoint, growth affects the shape and therefore the marketability of the fruit. From the scientific standpoint, growth governs the distribution of stomata. This distribution in turn is related to water loss and aeration.

In order to arrive at a quantitative measure of the growth of avocado fruits, about twenty-five Northrop fruits of varying sizes were marked on March 23, 1934, with parallel India ink lines (3 mm. apart) made by means of a rubber stamp. One pair of lines was placed near the tip end,[2] another at the location of the largest diameter, and a third near the stem end. All lines were perpendicular to the long (stem to tip) axis.

On May 21, 1934, an average marked fruit was 64 mm. long and 42 mm. wide at its greatest diameter. The average distance apart of the lines near

[1] The writer gratefully acknowledges the interest of Professor W. T. HORNE in these studies and the cooperation of Dr. L. J. KLOTZ in conducting the permeability tests.

[2] In this paper the popular terms "stem" and "tip" ends are used synonymously with the scientific terms "calyx" and "stylar" ends.

the tip end was 4.18 mm., near the region of largest diameter 5.27 mm., and near the stem end 6.22 mm. The region of least growth lies near the tip end, while that of greatest growth occurs between the region of the largest diameter and the stem end. This type of growth differs from that described by HAAS and BLISS (**4**) for date fruits, in which the region of greatest growth occurs at the region of attachment.

## STOMATAL DISTRIBUTION

When young fruits of the thin skinned varieties are about 1 inch in length, they show numerous stomata distributed over almost the entire surface. As the fruits develop, the stomata become separated by greater distances toward the stem than toward the tip end, as seen in figure 1.

FIG. 1. Mature Fuerte fruit showing distribution of stomata, which are farther apart toward stem end.

Figure 2 *A* shows a cross section of a stoma in the skin of a mature fruit of the Fuerte variety. The guard cells are seen closing the entrance to a

*A*

*B*

FIG. 2. Cross sections of skin of a mature Fuerte fruit: *A*, guard cells of a stoma in relation to primary stomatal cavity (×588); *B*, relation of a stoma to primary and secondary smaller cavities and spongy tissue (×141).

large stomatal cavity. The cuticle of the epidermis is relatively thick. The large stomatal cavity affords an excellent means for aerating the deeper lying tissues and for allowing moisture to escape rapidly from the large surfaces within the cavity.

The stomata gradually function less efficiently as the fruits approach maturity. Microscopic examination of a mature stoma in surface view frequently reveals a browning of the stoma and its eventual death. In some

cases the surrounding cells also die. Because of these changes there is less protection against drying out in these areas and the type of end-spots called speckles may result. Secondly, these areas offer agents of decay relatively easy access to the skin and pulp. In Fuerte fruit there is but little, if any, lenticel formation in the region of the stomata, so that mature fruits in a large measure have lost considerable of their previous protection against disease producing agencies.

*A*

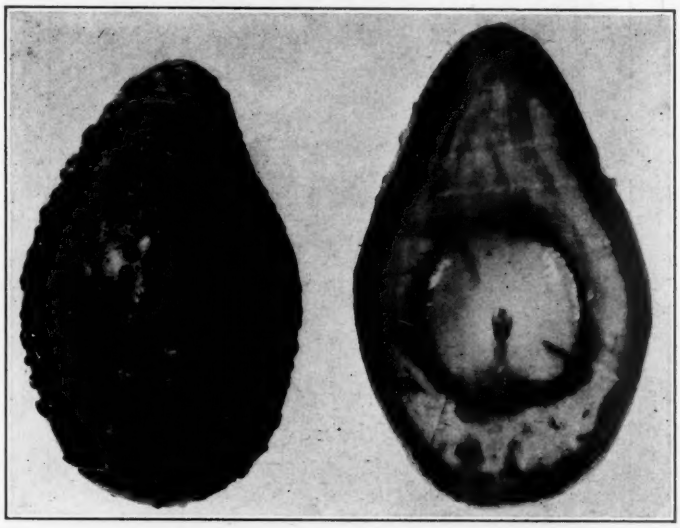
*B*

FIG. 3. Cross sections of skin of mature fruits of thick skinned varieties (×141): *A*, seedling; *B*, Premier.

Examination of the entire cross section of the skin of a mature Fuerte fruit (fig. 2 *B*), makes clear the relation of the guard cells to the large primary and the smaller, more deeply situated, secondary stomatal cavities. Much of the underlying tissue consists of spongy thin walled cells. The section also gives an idea of the relative thickness of the skin of fruits of thin skinned varieties.

In fruits of thick skinned varieties the death of the stoma is followed by varying degrees of cork formation, as a result of which lenticels are produced. Figure 3 *A* shows the excessive cork formation in the skin of a fruit of a seedling tree, while figure 3 *B* shows a lenticel in the skin of a fruit of the Premier variety. Such thick skins usually contain groups of stone cells. There is also considerable cork formation in the region below that previously occupied by the stoma. A brief, concise statement by STEVENS (7) describes such transformations of stomata into lenticels.

When the processes of gaseous exchange are greatly reduced, serious injury occurs, as may be seen in figure 4. Detached fruits of the Benik variety were fully coated with a paraffin of low melting point on February 24, 1932, and on March 18, 1932, some of the fruits were photographed. Figure 4 *A* shows the external appearance of the fruit. Figure 4 *B* shows



*A* *B*

FIG. 4. Effect of complete coverage of mature Benik fruits with low melting-point paraffin. *A*, raised blisters in the paraffin that are filled with a brown liquid; *B*, marked discoloration and decomposition of the pulp.

the fruit cut lengthwise, revealing the internal darkening and decomposition of the pulp tissue.

These artificially induced injuries suggest that the tendency of fruits of certain varieties to darken internally upon advanced maturity may be related to an inadequate gaseous exchange. The probable explanation of what happens is: (1) the fat content increases as maturity is approached, a greater gaseous supply and exchange thus being required; (2) the increasing

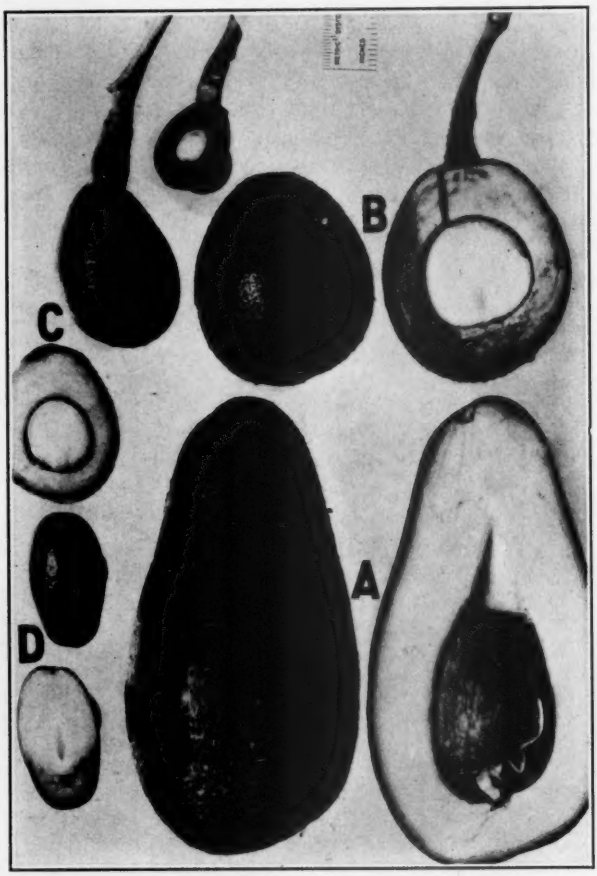



FIG. 5. Effect of immersing Fuerte fruits in distilled water for several days: *A*, mature fruit in which dead tissue surrounds the stomata, giving appearance of speckles. A slight darkening of the pulp occurs near periphery at tip end; *B*, *C*, *D*, immature fruits showing discoloration of skin toward tip and darkening of pulp beneath the injured skin.

fat content possibly retards the movement of gases in the tissues; and (3) the fruits of certain thick skinned varieties contain varying amounts of phellogen tissue with different degrees of permeability.

## Water relations

Water injury.—The immersion of Fuerte fruits of varying ages in distilled water for several days prevented a sufficiently rapid aeration of the tissues, as shown in figure 5.

The experiment was repeated with about twenty-five fruits of another thin skinned variety (Northrop). Fruits of various sizes were placed in liter beakers containing distilled water. These fruits, which had stems attached, were submerged in water that was frequently changed. At the end of two days no injury was observed, largely because the fruits were viewed from the stem end. After two and one-half days the fruits were taken out of the water, wiped dry, and thoroughly examined. Upon standing an hour or so in the dry condition, portions of the skin began to darken.

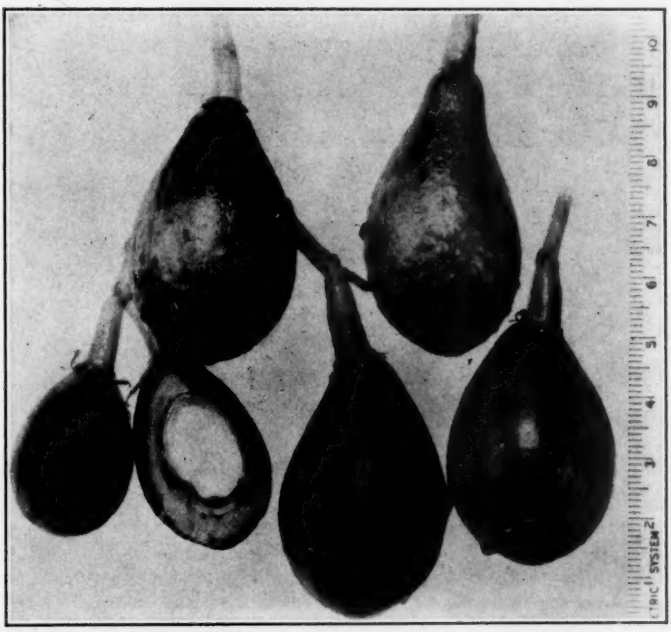


Fig. 6. Discoloration of skin of young, immature Northrop fruits (about 2.5 cm. diameter × 4 cm. long) by immersion in distilled water for 2 to 3 days. Photograph made one hour after fruits were dry.

Other experiments were conducted at room temperature to ascertain the period required for such damage to occur. After immersion for 8 hours, only one of the fourteen fruits showed injury after being dry four days, after 32 hours' immersion, five out of the eleven fruits showed injury after being dry 63 hours, after immersion for 48 hours every one of the eight large fruits showed injury after being dry 48 hours, and after immersion for three days each of eleven fruits showed injury after being dry 24 hours. In the long-necked fruits in the lot immersed for the longest period, severe checking[3] and darkening occurred on the neck portion.

Figure 6 shows the almost complete brown discoloration of the skin of the small Northrop fruits. After remaining in a dry condition overnight the discolored skin turned from brown to black and marked the location of sunken or depressed areas. The underlying pulp tissue was also darkened to varying depths. Frequently the stomata were included in small, sunken areas of dead skin, giving them the appearance of speckles.

The characteristic injury (called carpellary water injury) was evident at the extreme tip end where the fusion tissue of the ovary was weak. This fusion tissue forms a slight depression in the skin of the fruit at the tip end, as shown in figure 7.

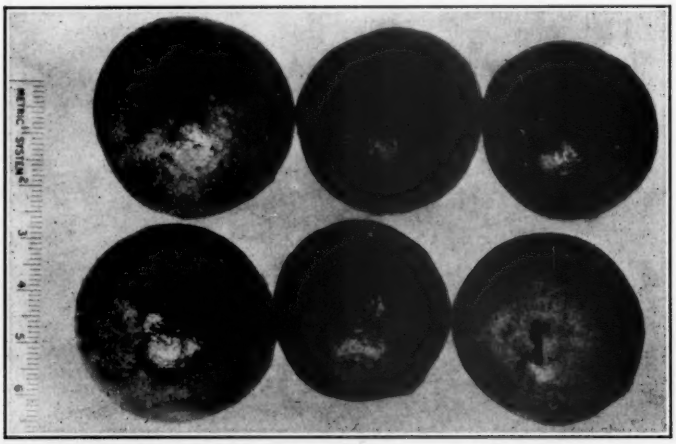


FIG. 7. Effect of immersion of half-grown (about 4 cm. diameter × 5 to 6. cm. long) Northrop fruits in distilled water for 2 to 3 days. Death of skin to varying depths into the pulp occurred at and surrounding the weak fusion tissue at the tip end. Photograph made one hour after fruits were dry. Apparent cavity where weak fusion tissue occurred is indicated by the "1" on control fruit in photograph.

[3] The term "checking" is used to designate small, visible, and also microscopic splits or ruptures in the skin of the fruit (HAAS and BLISS, 4).

Similar results were produced in fruits of various sizes still attached to the tree in the field by immersing them for periods of one to three days in jars of distilled water suspended from the branches. In this experiment considerable spotting of fruits resulted. The water apparently can penetrate the weak fusion tissue of the carpellary structure rather rapidly and then diffuse laterally after absorbing or pushing aside the bubble of air from the depression at the tip end. In penetrating at this point the water kills the tissue, the by-products of which are then free to kill or injure the adjacent tissue.

These results indicate the type of injury that may occur when fruit is continuously bathed with distilled water. After standing overnight the discolored skin and pulp of the artificially injured fruit becomes nearly black and a sinking of the tissue occurs in the injured areas as the affected tissues dry out.

Fruit on trees may become dry at the tip end, shrink, and crack, thus exposing the deeper tissue of the pulp to further drying and to other destructive agencies. Premature coloration and drying out of the weak fusion tissue of Northrop fruits at various stages of their development have been

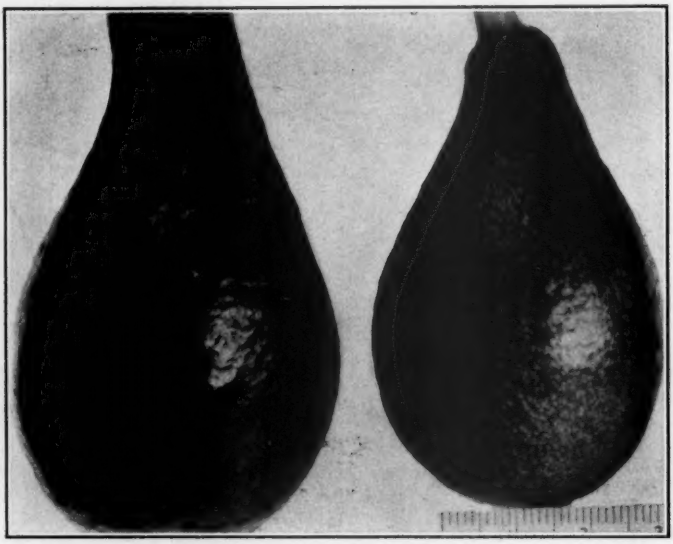
FIG. 8. Mature fruit of Blake variety affected with end-spot as a result of severe water deficit in the tree.

produced by withholding water for too long a time from the soil. Water applied to the soil after wilting appeared did not prevent the abscission of many young fruits. After several weeks the effects became more apparent on the more advanced fruit (about 4 cm. diameter × 7 cm. long). In addition to showing premature coloring, many of these fruits also showed some drying out and shrinkage.

The weak fusion tissue, in addition to absorbing water rapidly, also dries out readily when trees are under considerable stress for water. Fruits of the Northrop variety that were prematurely fully colored or even partially colored, showed, when under a water stress, a sunken, darkened area about this carpellary region (fig. 8).

Some of the more mature, but still fully green, Northrop fruits (about 4.5 cm. diameter × 8.5 cm. long) after being immersed nearly 3 days in distilled water and then wiped dry were photographed within an hour after being removed from the water. Figure 9 *A* shows such a water-injured fruit, together with a control fruit.

Dark areas in the skin and underlying pulp were scattered over much of the fruit. The dark region on the neck and that near the weak fusion tissue



*A* *B*

FIG. 9. *A*, water injury of a fully green, immature Northrop fruit (about 4.5 cm. diameter × 8.5 cm. long) produced by immersing fruit for nearly 3 days in distilled water; *B*, control fruit not immersed. Note checking of skin in neck portion of affected fruit.

were badly checked. As a result of these checks the dark areas soon lost water and began to sink. These checks are in some respects comparable with those studied and produced by HAAS and BLISS (**4**) by immersing date fruits in water. Overnight the water-injured fruit illustrated in figure 9 showed such increased injury that it was again photographed after 20 hours (fig. 10).

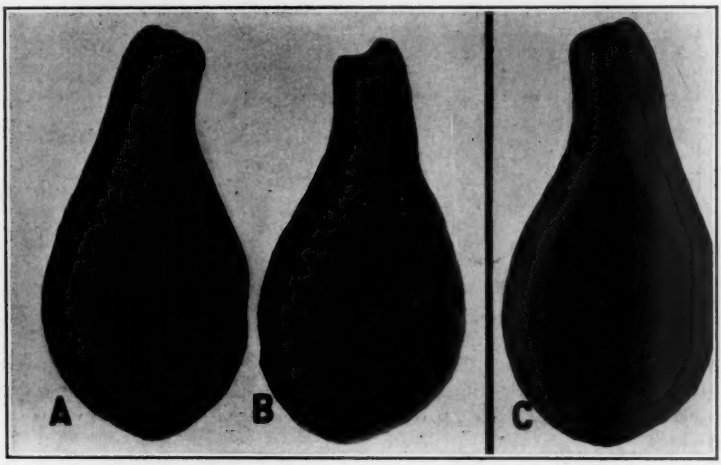



FIG. 10. Increasing effect of time on water injury in fruits shown in figure 9. Photograph was made about 20 hours after fruits were dry. *A* and *B* show exterior of the halves and advance of dark, sunken areas. Distance from mark in *A* to living skin indicates advance of injury during 20 hours. Checking of skin is shown on the neck in *B*. Two lines in *C* point toward water-soaked appearing pulpy tissue.

Water injury may be produced artificially in fruits of thick skinned varieties such as Tiger (fig. 11), as well as in fruits of thin skinned varieties such as Fuerte, Puebla, Blake, and Northrop.

In the laboratory an experiment was carried on with mature thick skinned fruits of the Spinks variety. Figure 12 shows the more violent type of water injury comparable to that which HAAS and BLISS (**4**) have designated as "tearing." The exposure to distilled water produced minute checks. When the fruit was allowed to dry the skin was drawn more tightly about the pulp and with further shrinkage was severely torn.

Attention thus far has been called to the artificial production of water injury in avocado fruits. The same type of water injury has been found under natural conditions in the field. On August 24, 1935, after an extended period of foggy mornings, nearly 2 inches of rain fell at the Citrus

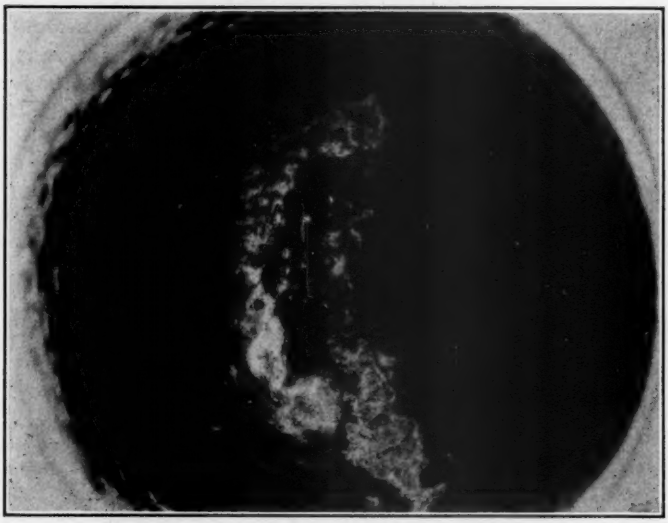


FIG. 11. Checking of skin at tip end of mature Tiger fruit produced by immersing in distilled water from April 9 to 16, 1934 (fruit was attached to a tree in the field). Subsequent drying of skin after the fruit was detached tended to increase visibility of the checks.

Experiment Station. Light sprinkles of rain occurred on August 26 and on several other previous days. On August 29, 1935, many fruits were found on trees at the Citrus Experiment Station that showed injury as a result of checking. Figure 13 shows affected fruits that were photographed under water after rubbing talcum powder into the checks in order to improve their visibility.

These results are of interest because FRAZIER (2) has stated, regarding the cracking of tomato fruits, that the most consistent effect obtained was the cracking after the applications of water to the fruits or to the soil.

Thick skinned fruits on trees grown in soil kept very wet over a prolonged period because of heavy rains may abscise in large numbers. It is possible that such abscission follows a reduced oxygen content of the soil solution which has injured some of the younger roots.

PERMEABILITY OF SKIN.—The relative permeability of stem and tip halves of fruits was investigated by the methods employed by HAAS and KLOTZ (5) in determining the permeability gradients in citrus fruits.

Two lots each consisting of five mature Puebla fruits (somewhat changed from green to mahogany purple color, but firm) were collected November 1,

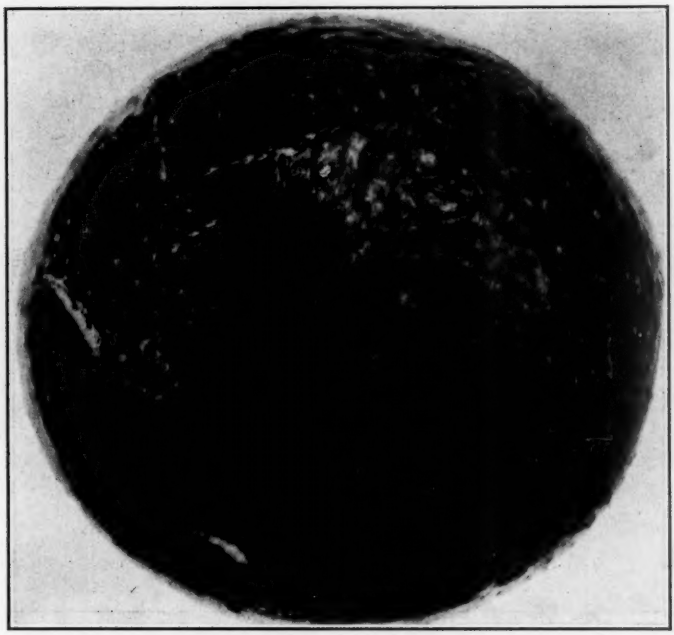


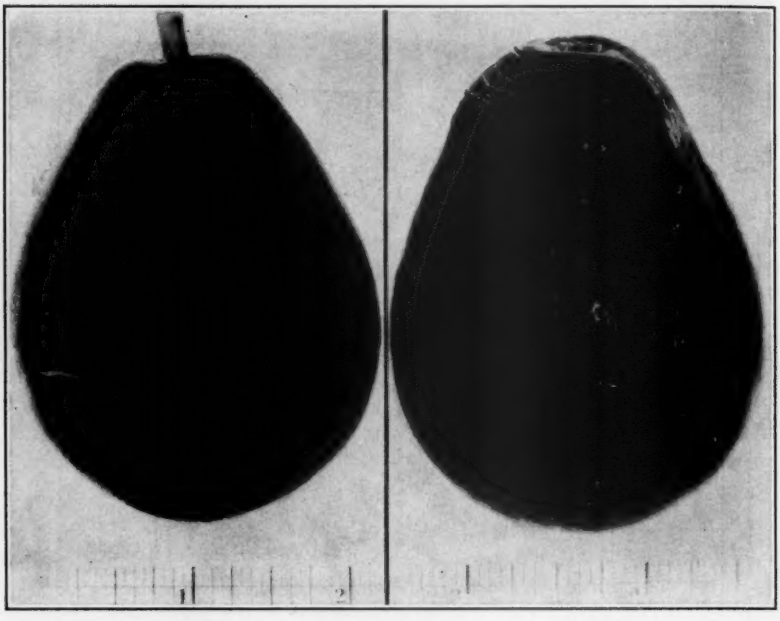
FIG. 12. Tearing type of water injury in fruit of Spinks variety produced artificially in the laboratory by immersing tip half of mature fruit in distilled water at room temperature from March 30 to April 6, 1934. Minute checks gradually enlarged until the tearing of the skin resulted. Drying of slightly torn skin exaggerates torn condition.

1932, at Riverside, California. For the permeability tests it was necessary to secure equal surface areas on both halves of the fruit. Equal lengths of ½-inch adhesive tape were placed on the stem half of one lot and on the tip half of the second lot. In both cases the entire fruit was momentarily immersed in paraffin of low melting point. The tape was then removed and the fruits immersed in 700 cc. of tap water for 53 hours.

The data in table I show that the permeability of the skin of the tip halves is greater than that of correspondingly equal areas of skin of the stem halves.

A greater number of stomata occur at the tip than at the stem end (fig. 1). In fruits of the thick skinned varieties the corky lenticels may be much larger than the stomata which they have replaced and may be so close to one another as to show a coalescence.

Since stomata and lenticels are supposed to function chiefly in gaseous exchange and at the same time to permit a loss of water, the greater number



*A* *B*

FIG. 13. Water injury of thin skinned varieties of fruits as it occurs in the field after periods of heavy fogs or rains during certain stages in maturation of the fruit: *A*, fruit of Mexican seedling tree; *B*, fruit of Mexicola variety.

at the tip end would permit a more rapid exudation of substances that catalyze the reaction involved in the reduction of $KMnO_4$. As has been shown, the permeability of the skin of Puebla fruit is greatest in the tip half, whereas that of citrus fruit (5) is greatest in the stem half.

TABLE I

TIME (MINUTES AND SECONDS) REQUIRED TO MATCH 25 CC. $KMnO_4$ STANDARD

| PORTION OF FRUIT | STANDARD (3 DROPS $KMnO_4$ PER 50 CC. TAP WATER) | | STANDARD (1 DROP $KMnO_4$ PER 50 CC. TAP WATER) |
|---|---|---|---|
| | *min. and sec.* | *min. and sec.* | *min. and sec.* |
| Stem half (3 sq. in. not paraffined) | 5:50 | 5:35 | 1:55 |
| Stem half (3 sq. in. not paraffined) | 1:50 | 1:45 | 0:30 |

RATE OF WATER LOSS.—Lots of three to thirteen mature fruits (depending on the fruit size) were obtained at Riverside on September 12, 1931. The rate of water loss was determined at room temperature in two ways: (1) by cutting the fruits into portions and placing the cut surface on glass plates coated with boric acid powder, and (2) by paraffining the stem or the tip half of the fruit.

After certain periods the water loss was calculated as a percentage of the original fresh weight. The results in table II show that regardless of the method used, the water loss is consistently greater in the tip than in the stem half, and that it is intermediate in the middle portion of the fruit.

TABLE II

WATER LOSS THROUGH VARIOUS PORTIONS OF SKIN OF AVOCADO FRUITS

| VARIETY | PORTION OF FRUITS | WATER LOSS AS PERCENTAGE OF ORIGINAL FRESH WEIGHT | | |
|---|---|---|---|---|
| | | 28 HOURS | 68 HOURS | 95 HOURS |
| | Fruits cut into halves | | | |
| | | % | % | % |
| Mexicola | Stem halves | 5.66 | ..... | 17.52 |
| | Tip halves | 6.83 | ..... | 21.25 |
| Mexicola | Stem halves | 6.13 | ..... | 17.83 |
| | Tip halves | 6.79 | ..... | 21.70 |
| Blake | Stem halves | 6.47 | ..... | 18.95 |
| | Tip leaves | 7.75 | ..... | 23.25 |
| Blake | Stem halves | 6.80 | ..... | 20.40 |
| | Tip halves | 8.20 | ..... | 23.55 |
| Blake | Stem halves | ..... | 14.00 | 19.53 |
| | Tip halves | ..... | 16.15 | 22.56 |
| Blake | Stem-end thirds | ..... | 16.26 | 22.49 |
| | Middle thirds | ..... | 17.94 | 24.80 |
| | Tip-end thirds | ..... | 18.87 | 26.42 |
| One-half of fruit paraffined | | 69 hours | 235 hours | 452 hours |
| Blake | Stem halves | 4.51 | 12.44 | 27.72 |
| | Tip halves | 5.33 | 14.87 | 34.12 |
| Fuerte | Stem halves | 2.52 | 7.13 | 14.90 |
| | Tip halves | 4.58 | 12.05 | 24.29 |
| One-half of fruit paraffined | | 48 hours | 237 hours | 432 hours |
| Benik | Stem halves | 3.84 | 13.89 | 26.08 |
| | Tip halves | 4.36 | 15.65 | 29.25 |
| Puebla seedling | Stem halves | 3.04 | 10.73 | 19.08 |
| | Tip halves | 3.89 | 14.65 | 25.71 |
| One-half of fruit paraffined | | 53 hours | 292 hours | 502 hours |
| Benik | Stem halves | 3.66 | 14.26 | 22.34 |
| | Tip halves | 4.04 | 16.09 | 25.12 |

The effect of fruit size on the rate of water loss from fruits kept at room temperature was next studied. Immature avocado fruits were obtained at Riverside on April 14, 1930, and their original water content was determined by drying similar samples at 80° C. in a well ventilated oven. The original fresh weight per fruit (table III) gives some idea of the fruit size.

As shown in table III, the smaller fruit (younger) of a given variety lost

TABLE III

EFFECT OF FRUIT SIZE ON RATE OF WATER LOSS

| VARIETY | NUMBER OF FRUITS | ORIGINAL FRESH WEIGHT PER FRUIT | ORIGINAL WATER CONTENT | LOSS OF ORIGINAL FRESH WEIGHT | |
|---|---|---|---|---|---|
| | | | | 48 HOURS | 91 HOURS |
| | | *gm.* | % | % | % |
| Blake | 29* | 0.5641 | 84.77 | 46.58 | 73.30 |
| | 20 | 1.2153 | 84.82 | 40.34 | 68.03 |
| Caliente | 38* | 0.3196 | 84.84 | 60.27 | 79.93 |
| | 21 | 0.8125 | 84.74 | 47.61 | 73.40 |
| Mexicola | 29* | 0.4537 | 86.27 | 48.79 | 77.46 |
| | 8 | 2.5785 | 86.32 | 30.95 | 57.15 |
| Tiger | 39* | 0.3755 | 86.09 | 51.69 | 78.95 |
| | 17 | 1.6012 | 86.19 | 38.64 | 67.65 |

* Smallest fruits of the two lots.

water more rapidly than the larger (older) fruit of the same variety, although both lots of fruit contained the same percentage of water in the original fresh weight. This difference in water loss from fruits that depends on the fruit size or age may be due to several causes, two of which are: (1) there are fewer stomata per unit area of skin as the fruit increases in size (fig. 1); (2) the larger fruits have less surface exposed per unit volume, a condition shown by HAAS (3) to be a factor in water loss from young citrus fruits.

## Summary

1. The growth of young Northrop fruits was found to be greatest in the stem end and progressively less toward the tip end.

2. The region of greatest growth in a fruit may be found by observing the distribution of stomata. In young fruits the stomata are rather uniformly distributed, but as growth proceeds, the greater growth toward the stem end separates the stomata more than in other regions of the skin.

3. Stomata in the skin of mature fruits afford a rapid means of gaseous

exchange and transpiration; however, under certain conditions they also afford a rapid means of wide-spread fungus distribution within the tissues of the skin and pulp. In thick skinned varieties the death of a stoma is usually followed by the formation of lenticels, but in the thin skinned varieties such as Fuerte there is little lenticel formation.

4. Reduction in the gaseous exchange results in serious damage to the quality of the fruits.

5. Immersion of fruits of the Fuerte variety in distilled water injured the tissue in the skin of the mature fruit in the region of the stomata, and especially injured the skin tissue at the tip end. The pulp underlying the damaged skin is variously injured, depending on the length of the period of immersion.

6. Immersion of nearly mature Northrop avocado fruits in distilled water for three days produced: (1) checking in the neck portion at the stem end; (2) carpellary water injury at the tip end, where slight checking also may occur; and (3) injury or death of tissue in the region of the stomata. The more immature the fruits the more rapid the injury, with the exception of checking in the neck portion which occurred primarily in the more mature fruits with the prolonged immersion. These injuries were produced both in the laboratory and in the field while the fruits were still attached to the tree. Similar injuries can occur to fruits under natural conditions in the field.

7. Carpellary water injury was produced in fruits in the field by withholding water for too prolonged a period while the fruits were young. Abscission of some of the small fruits occurs while some of the large ones dry out in the region of the weak fusion tissue of the ovary. The lack, as well as an excess, of water in this region of the fruit brings about somewhat similar effects. Such injury is usually followed later by checking, drying, shrinking, and decay.

8. Checks located only at the tip end were produced in thick skinned, mature fruits of the Tiger variety when immersed for seven days in distilled water while the fruits were still attached to the tree. Tearing of the skin of a nearly mature fruit of the Spinks variety resulted when the tip half of the fruit was immersed for seven days in distilled water in the laboratory.

9. The permeability of the skin of the tip halves of Puebla fruits is greater than that of correspondingly equal areas of skin of the stem halves.

10. The rate of water loss from the tip halves of fruits exceeds that of the stem halves.

11. Small fruits lose water more rapidly than larger (older) ones of the same variety.

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# EFFECTS ON TREES OF AN ILLUMINATING GAS IN THE SOIL[1]

CARL G. DEUBER

## Introduction

Problems concerned with trees injured by illuminating gas accidentally leaking into the soil may be said to have commenced in 1807 when the first public street gas lighting system was installed in Pall Mall, London (**10**). Although various phases of the problems presented when illuminating gas is seeping into soil in which the roots of plants are growing have been investigated by botanists, chemists, and others for at least seventy years, no general agreement has been reached upon the exact manner in which vegetation is injured, killed, or otherwise influenced. A brief survey of the literature consulted is given to establish the present status of the general problem.

NEUMAN in 1842, according to GIRARDIN (**12**), described the poisoning of elm trees by gas. By 1864, GIRARDIN, in Lille, France, was studying the soil for the cause of severe injury to Italian poplar trees located near a leaking gas conduit. About this time injuries to street trees were being reported from a number of European cities. The cause of the injury, and death of shade trees on several of the chief thoroughfares in Berlin brought about an extended controversy. This was described by LAISSUS and HEIM (**16**). Some thought that gas in the soil was responsible; others thought that unfavorable conditions of water supply, nutrition, high temperatures, and other factors were chiefly responsible for the conditions of these trees. It was suggested that the soil around the roots of the trees should be inclosed by impervious cylindrical walls to avoid the action of illuminating gas, from leaking conduits, on the trees. In 1900, WEHMER (**25**) described the injury of elm trees in the vicinity of a gas leak in Hanover, Germany, and included an illustration showing the leafless condition of the tops of the trees and the sloughing off of large patches of the basal bark. In 1903 (**18**), at Yonkers, New York, considerable trouble was experienced owing to gas injuries upon shade trees. The writings of STONE (**21, 22, 23, 24**), from 1906 to 1916, gave evidence of the frequent instances of injuries to trees in New England which were attributed to the escape of illuminating gas in the soil. In recent years, in the United States field cases of gas in the soil affecting tree growth unfavorably have rarely been reported in the scientific literature. Accidental occurrences of this type are more frequent than is generally supposed. When they involve a conspicuous shade tree or extensive injury to a number of trees in a small locality the necessity for information upon the factors involved, accurate diagnosis, and possible remedial measures become urgent.

[1] Contribution from the Osborn Botanical Laboratory, Yale University.

Investigative work with trees experimentally subjected to illuminating gas has covered a variety of the aspects of the general problem. LAISSUS and HEIM (**16**) describe one experiment by POSELGER in the 1860's, in Berlin, in which eight young trees planted in a wooden box were subjected to a flow of gas into the soil for three hours a day during forty days. No ill effects to the trees were observed and the conclusion was that gas in the soil was not injurious to trees. The toxic nature of the gas in Berlin was soon afterward established by KNY (**15**), and SPÄTH and MEYER (**20**). Differences in the susceptibility of various species of trees to injury by gas were noted by these investigators, and by WEHMER (**26**), DOUBT (**7**), and others. The toxicity of illuminating gas in the soil was attributed to residues of "brown oily substances" as well as high contents of sulphur and ammonium salts by GIRARDIN (**12**). BOEHM (**1**) associated the injurious action of gas with the "tarry products" left in the soil. BRIZI (**2**), EHRENBERG (**8**), EHRENBERG and SCHULTZ (**9**), SORAUER (**19**), and SCHOLLENBERGER (**17**) considered the displacement of oxygen in the soil atmosphere the chief cause of injury to vegetation. HARVEY and ROSE (**13**) thought ethylene was the most harmful constituent remaining in the soil in a gaseous state. WEHMER (**27**) emphasized hydrocyanic acid as the chief toxic agent. HITCHCOCK, CROCKER, and ZIMMERMAN (**14**) found that while the removal of hydrocyanic acid greatly reduced the toxicity of one illuminating gas it did not eliminate the injurious effects of the gas in the soil. Detailed descriptions of the symptoms and the field conditions associated with shade trees injured in various ways were made by STONE (**21, 22, 23, 24**). The writer (**3, 4, 5, 6**) reported injurious as well as stimulative phenomena which were observed when small trees or tree parts were exposed to illuminating gas.

The accurate determination of injuries to established trees, directly attributable to gas in the soil, is attended with many difficulties. Three major factors involved in determining the cause of injuries are: the gas, the soil environment, and the tree. Each of these is complex and highly variable, and the diverse combinations that may and actually do result present many confusing field problems. Frequently the symptoms of gas injury in a tree are distinguished with difficulty from those resulting from other unfavorable conditions for growth. Conditions such as a hard packed surface soil, and coverings of impervious paving, which restrict aeration of the soil, make the solution of the problem more difficult. Illuminating gases are of different types depending on the process of manufacture. The composition of one gas may vary widely from time to time. Differences in the chemical composition of manufactured gases may account for the more severe action of gas leaks on vegetation in one community than in another. Gas from a modern manufactory is subjected to purification processes in the recovery of by-products and in the removal of ingredients known to

have a corrosive action on metal pipes so that the constituents held chiefly responsible for the toxic action of gas in the soil during the past and from older manufacturing plants may not be those responsible for the effects of all illuminating gases. In an unpublished work the writer tells of three types of currently used manufactured gas. He here indicates that the epinastic response of tomato plant leaves varies with the respective gases when these gases surround the roots. The extent of a gas leak, the rate of flow of the gas, the quantity passing into the soil, and the time over which it acts are additional conditions of importance in determining the influence of illuminating gas on vegetation. It is with the latter features of rate and volume of gas flow that the present study is concerned. An attempt was made to establish for the gas used, and under the conditions imposed, quantitative limits for the amount of gas required to kill small trees when the illuminating gas was passed into the soil about their roots.

## Experimentation

During the spring and summer of 1934 the following tests were made for the purpose of recording the influence of various rates of flow and quantities of a typical manufactured gas supplied to the soil in which three-year-old American elm trees were growing. The trees had been grown in clay pots and in April were transplanted to cans holding approximately 1 liter of soil. A layer of sand was placed on the bottom with a glass tube extending from the sand layer to beyond the rim of the cans for the introduction of the gas at the bottom of the soil and the root system. Upon a layer of loam soil the root mass of the potted trees was placed and additional soil sifted and tamped around the sides. Thirty trees were prepared in this manner and given regular greenhouse care.

Gassing of the soil of the trees took place outside a ground level window with rubber and glass tubing reaching from a laboratory gas outlet through a wet meter or a calibrated flow-meter to the bottom of the soil in the cans. The gas was of the coke oven type as supplied by the New Haven Gas Light Company. For the period covered by this study the average composition of this gas, together with the percentage of each component, except for naphthalene which is recorded in grains per 100 cu. ft., is given as follows:[2]

| | |
|---|---|
| Carbon dioxide | 1.70 |
| Illuminants | 3.00 |
| Oxygen | 0.20 |
| Carbon monoxide | 8.70 |
| Hydrogen | 51.00 |
| Methane | 25.90 |

[2] The writer is indebted to Mr. W. A. Fitzsimmons, Superintendent of Production of the New Haven Gas Light Company for this analysis.

| | |
|---|---|
| Nitrogen | 10.00 |
| Naphthalene | 3.50 |
| Sulphur | trace |
| Hydrocyanic acid[3] | —— |

Between May 2 and August 7, 1934, twenty of the elm trees were subjected to a gassing of the soil about their roots. In table I brief descriptions of ten of the trees during and following the gassing treatment are reported. Later, additional data will be reported in regard to trees subjected to gas in the soil when the surface of the soil was covered with paraffin. Ten trees were grown through the summer as controls. Both the control and the gassed trees were placed in shallow trenches in the soil out-of-doors over winter so that the effects of the gas treatment upon the trees during the next season could be observed.

When injury to a tree occurred from gas passing into the soil the earliest symptoms observed were chlorosis of the leaves and defoliation. The conditions existing in the roots were not determined because it would have required the disturbance of the soil structure, which might have interfered with subsequent effects of the gas or its residues upon the trees. Chlorosis generally involved the leaf margins first and sometimes proceeded no farther. In a few cases, entire leaves quickly turned yellow and shriveled or abscissed without shriveling. Saturation of the soil by rain during the gassing of trees no. 3 and 7 was associated with this rapid type of chlorosis and defoliation. Usually, the lowermost leaves on the main stem or larger branches became chlorotic and abscissed before the younger upper leaves. When complete defoliation of some or all of the branches occurred the buds either remained unaffected or they shriveled and darkened in color as did the distal portions of the branches and twigs which became dry and brittle. Frequently the distal one-third or one-half of a branch and its buds died while the basal portion and its buds remained visibly unaffected. Shortly after complete defoliation trees no. 4 and 6 began producing vigorous new shoots from buds below the injured or dead portions of their branches.

During the summer of 1934 it was possible to class but one tree, no. 2, as killed. By the next spring no. 7 was dead. All the others lived and made some growth during the 1935 season. The older stems of some of the trees had died for a part of their length, but on the uninjured part new shoots developed and made vigorous growth. When the roots of all the trees were examined on October 10, 1935, they showed relatively few abnormalities. It was found that when tree no. 6 was subjected to 864 cu. ft. of gas, in 1934, the distal half of the tap root was blackened and brittle and many of the fibrous roots tore off readily when the soil was removed, but several lateral roots had an abundance of healthy fibers. At this time, the stem of

[3] The laboratory reports showed no hydrocyanic acid present.

## TABLE I

SYMPTOMS OBSERVED IN TEN YOUNG ELM TREES GASSED IN CANS OF SOIL

| TREE NO. | DATE | RATE OF GAS FLOW | TIME GASSED | TOTAL GAS SUPPLIED | SYMPTOMS |
|---|---|---|---|---|---|
| | | *cu. ft./hr.* | | *cu. ft.* | |
| 1 | May 2 | 4.2 | 11 hrs. | 46.2 | Gradual defoliation of lower leaves except one lower branch. Defoliation complete by August 29 except for the normal lower branch. Buds dried on two branches; others normal |
| 2 | May 4 | 4.2 | 7⅔ hrs. | 32.2 | Yellowing of margins of lower leaves during the first three months. In August, complete defoliation. Roots injured; buds very small. Dead, 1935 |
| 3 | May 18 | 1.5 | 5 days | 180.0 | Considerable defoliation of lower leaves on fifth day. Some marginal chlorosis. A number of leaves retained in a functional state until October. Buds well filled |
| 4 | June 20 | 1.5 | 6 days | 216.0 | General defoliation on fifth day of gassing, remaining leaves turned yellow and shriveled. In a month, 8 new shoots developed. By end of August, foliage on new twigs about mature and normal. Dead buds at ends of older branches |
| 5 | July 13 | 0.85 | 15 days | 306.0 | Slight chlorosis of lower leaves in 3 days; partial defoliation in 62 days. Stem at ground line became swollen; outer bark separating in long plates. Inner bark soft. Buds well filled at end of growing season |
| 6 | July 28 | 7.2 | 5 days | 864.0 | Slight defoliation in two days, heavy defoliation next two days. In a month 26 new shoots were developing from buds 2 to 4 inches below bare tips of older branches. New foliage remained normal |

TABLE I.—(*Continued*)

| Tree no. | Date | Rate of gas flow | Time gassed | Total gas supplied | Symptoms |
|---|---|---|---|---|---|
| | | *cu. ft./hr.* | | *cu. ft.* | |
| 7 | Aug. 3 | 0.6 | 11 days | 158.4 | Partial defoliation and chlorosis in 4 days. Complete defoliation in 11 days. Buds and twigs dried and became very brittle. Roots severely injured by October. Dead, 1935 |
| 8 | Aug. 7 | 5.5 | 1 hr. | 5.5 | No visible injurious effects |
| 9 | Aug. 7 | 25.0 | 12 min. | 5.0 | In three weeks the foliage appeared a trifle lighter green than that of control trees, otherwise no ill effects observed |
| 10 | Aug. 7 | 20.0 | 30 min. | 10.0 | No injury observed. |

tree no. 5 had become swollen at the ground line. While a deep crack into the wood had formed it was being covered by the growth of callus tissue and the bark was in unusually thick plates over the base of this stem. Observations of the condition of the stems and buds of the gassed trees at the close of the 1934 season proved to be a reliable guide in determining the severity of the injury sustained. Injury to the trees became evident during and following the actual exposure of the roots to gas in the same growing season. Residual effects of the gas treatment in the soil or in the trees did not appear to accumulate and bring about more severe injury or lethal effects the next year.

In addition to the foregoing tests, three elm trees in cans, prepared as previously described, were treated by passing 36, 14, and 7 cu. ft. of gas into the soil and immediately sealing the soil surface with a layer of melted paraffin. The surface of the soil of one control tree was similarly sealed and the control trees of the previous tests served as checks of the behavior of the trees in normal soil with a free surface. Within ten days after the treatment of the soil of one tree with 36 cu. ft. of gas the characteristic "ethylene effect" of leaf epinasty appeared. Epinasty was most evident in the lower leaves, and less evident in the upper young leaves. Gradually the leaves of this tree became very dry and hard, the older leaves became brown from the margins to within 1 cm. of the midveins. The tree in soil receiving 14 cu. ft. of gas lost two-thirds of its leaves and exhibited marked chlorosis of the remaining leaves. The tree in soil through which 7 cu. ft. of gas had been passed continued normal in appearance except for a slight chlorosis at the bases of three leaves. The leaves of the control tree with the soil surface sealed became somewhat dry and hard. One month after

the gas treatments these trees were removed from the cans for an examination of the roots. The soil was very dry and the small fibrous roots broke readily when the soil was being removed. Secondary roots had some areas of outer bark sloughing off. These features were common to the roots of the control tree with the soil surface sealed as well as of the gassed trees. During 1935 these trees made normal growth of tops and roots.

## Discussion

The experimental results in this study in many respects follow the observed conditions and the known facts of a number of field cases of injury to established shade trees by illuminating gases leaking into the soil, which the writer has followed during the past six years. Rapid killing of a shade tree within a few days or a few weeks has been seen occasionally, but the more numerous cases are those in which chlorosis of the foliage on a portion of the tree and partial defoliation is subsequently followed by the drying out and death of uppermost twigs, and the dying of some branches and not of others. This process of gradual injury, although making for an unsightly tree and a hazard on streets because of the brittle branches likely to fall at any time, may continue for three or more years. The latter cases are the most confusing to diagnose, especially when the source of the gas in the soil is no longer evident and the tree is subject to serious insect and fungus attacks as a result of its lowered vitality, exposed branch stubs, loosened bark, and similar features associated with such trees.

With these young elm trees, two of the twenty were completely killed by the spring of the next season after the gassing. While some of the trees, those subjected to 5 and 10 cu. ft. of the coke oven gas, exhibited no signs of injury, those gassed with 32 to 864 cu. ft. of gas showed chlorosis of leaves, partial or complete defoliation, and the death of terminal parts of branches. In two instances, defoliation was soon followed by the development of latent buds on the stems below the injured part. In the following season many instances of partially killed stems were observed but new shoots were also produced and made excellent growth. Complete recovery would be expected with these young trees, based on their behavior the season following the gassing. Complete recovery in a larger tree would not be expected because of its age and the loss of so many branches from its top, regardless of the formation of new shoots.

Flowing 5 to 864 cu. ft., or 141.5 to 24,451 liters, of coke oven gas into one liter of soil for a period of twelve minutes to fifteen days was thought to embrace sufficiently drastic experimental conditions to produce rapid and definite lethal effects in these small elms. The results of the tests made it necessary to abandon the objective to determine even approximately the volume of this gas required to flow into the soil to kill these trees. Neither

the conditions of the tests nor the physiological action of the toxic ingredient or ingredients permitted assigning definite values to the amount of this illuminating gas to produce unmistakable lethal effects in a short period of time. A review of the experience of others in experimentally subjecting trees to illuminating gases in the soil will add to the interest in the explanations offered for the results of this investigation.

KNY (**15**) supplied illuminating gas at the rate of 418.5 cu. ft. per day through four burners in the soil at the base of a twenty-year-old maple tree. The gas flowed from early July to January and involved an enormous volume of gas. A neighboring *Evonymus* bush showed withering of leaves and defoliation early in September; a nearby elm tree exhibited signs of injury; while the maple defoliated early in the fall. The following spring the bush and the two trees were dead. Into the soil beneath a linden tree 380 cu. ft. of gas were passed daily. This tree, although severely injured, was able to produce small pale leaves the next spring. SPÄTH and MEYER (**20**) found that 25 cu. ft. of gas per day, in July, killed the roots of established trees in a few days, but in the winter when the roots were dormant the trees were much more resistant to injury by twice this rate of gas flow. SHONNARD (**18**) found that 1.07 cu. ft. of gas per hour added to the soil of a potted lemon tree caused chlorosis of the leaves in five days and defoliation in eight days. EHRENBERG and SCHULTZ (**9**) subjected two-year-old linden trees to rates of gas flow into the soil of 0.2 to 2.7 liters per hour for a month. Partial defoliation was the chief feature noted, and when the trees were transplanted to fresh soil they recovered.

Work at the University of Chicago constituted a major contribution in the United States upon the plant physiological effects of illuminating gas. HARVEY and ROSE (**13**) recorded a variety of experiments with seedlings and potted plants exposed to ethylene-air mixtures, and to illuminating gas. High concentration of illuminating gas brought about rapid killing of the roots, and the chief symptom was death. This same gas flowing slowly through the soil in which woody plants were growing brought about a stimulation of the roots which resulted in the development of new tissue. When "water gas" of the Chicago Gas Light and Coke Company was distributed at the rate of 1.5 liters per hour in the soil beneath an *Ailanthus* tree on the campus from July 3 to September 2, leaves of some of the younger shoots wilted in eleven days. Subsequently, these and a few more leaves shriveled and died but no general effect over the entire tree was observed until early defoliation occurred in September. In October, the tree was dead and was characterized by a general dryness of the tissues. DOUBT (**7**) demonstrated the sensitivity of the tomato plant and other plants to low concentration of ethylene and illuminating gas in the atmosphere, and tested the responses of small potted trees to gas passed into the soil. With eight trees of different species

treated forty to ninety days with 60 to 180 liters of gas in the soil, the foliage was little changed by the treatments. Swelling of the basal portion of the stems, cracking of the bark, and the production of proliferation tissue on underground parts were frequent symptoms. The most severe injury was exhibited by an American elm tree subjected to 180 liters of gas during ninety days. This tree lost half of its leaves, the bark at the base of the stem cracked open, and at the conclusion of the treatment the underground parts were dead. The conclusion was reached that the killing of trees by illuminating gas is a very slow process which may take months or years.

Previous studies by the writer (**3, 4, 5**), in which woody plants and their parts were subjected to a "mixed" illuminating gas, and in some instances to ethylene led him to conclude that there were three classes of plant physiological response to this illuminating gas: First, stimulation, such as acceleration of early development of latent buds, epinastic growth of leaf petioles, hypertrophy of lenticels, and proliferation of root parenchyma cells. Second, injury, such as inhibition of bud development (particularly terminal buds), dwarfing of leaves, loss of rigor by leaves, chlorosis, drying of portions or of entire leaves. Third, killing effects, which differ primarily in degree from those of injury. They were made evident in dormant trees by a blackening and drying out of buds without any sign of normal development, drying and wrinkling of the bark, drying out and brittleness of the stem and root wood, and when rapid, no hypertrophy of root lenticels or proliferation of parenchyma cells of the root. The type and the degree of the physiological response of these small trees varied with the time of exposure to the gas, the volume of the gas, the condition of the tree (dormant or growing), and the organ or part of the tree exposed to the gas.

HITCHCOCK, CROCKER, and ZIMMERMAN (**14**) found that when gas of the Westchester Lighting Company was introduced into the soil of small potted willow, maple, cherry, and silver bell trees at volumes of 1 and 4 cu. ft. for a period of thirty minutes, the larger volume caused the greater injury. Four cubic feet of the gas brought about severe injury to the roots with no ill effects on the aerial parts except in willow and maple. All of the woody plants, including privet, recovered in three to four weeks after the gassing with 4 cu. ft. of this gas. Ten cubic feet of this gas killed the five species of woody plants.

While the literature regarding the experience of other workers, who subjected trees to illuminating gases in the soil, does not permit concise generalization, it is evident that in most of these studies rapid lethal effects were not reported. Relatively large volumes of gas were required to seep into the soil for a considerable period of time before the death of entire trees occurred. Leaf, stem, bud, and root injuries frequently occurred, and, if the gas flow was of sufficient magnitude and continued long enough, death

of the entire tree resulted. Injury to tops and roots in a number of cases was followed by recovery. A possible exception may be seen in the work of HITCHCOCK *et al.* (**14**) in which lethal effects on woody plants were observed when 10 cu. ft. of the Westchester Lighting Company gas was passed into the soil of small potted trees. The highly toxic nature of this gas was ascribed to the presence of hydrocyanic acid. When this acid was removed from the illuminating gas, twenty to twenty-four times more gas was required to produce the same type and degree of injury induced by the unscrubbed gas. Removing hydrocyanic acid, however, did not make this gas non-toxic.

Since the coke oven gas used in the present study was freed of hydrocyanic acid by an efficient scrubbing process at the manufactory it is logical to seek the explanation for the effects produced in the elm trees in the action of other constituents.

Experiments performed at this laboratory in which a "mixed" illuminating gas as well as ethylene and air mixtures were used, led the writer to conclude that it is possible to ascribe the responses of the elm trees gassed in the soil with coke oven gas to ethylene, or compounds with a similar physiological action on woody plants. In the complex composition of a commercial illuminating gas there may be a number of ingredients which will affect woody plants as ethylene does. The earliest symptoms of controlled soil-gassing in the elm trees were chlorosis and defoliation. Unpublished work done at this laboratory with ethylene in concentrations of 1 to 50 per cent. in air held about the bases of rooted cuttings of privet or the washed roots of small oak trees caused chlorosis, defoliation, and the drying out of the tops of woody plants. It is concluded therefore that ethylene, or gaseous ingredients of similar physiological action on plants, can explain the symptoms observed when relatively large volumes of the coke oven gas employed in this investigation are passed into the soil in which small elm trees are growing.

## Summary

1. This investigation is concerned with a description of the symptoms observed when relatively large volumes of a coke oven type of illuminating gas were flowed into the soil in which young elm trees were growing, and with the probable explanation of these symptoms.

2. Five or ten cubic feet of this coke oven gas applied to the roots of elm trees produced no visible signs of injury.

3. When 32 to 864 cu. ft. of gas were used, a variety of injurious symptoms were observed in the trees. Chlorosis and defoliation were the chief early symptoms, followed, in some cases, by the drying out of the apical portions of the stems. Roots sustained injury but the sequence of this injury and its extent were not determined with these trees in undisturbed soil. Epinasty of leaves was observed following gassing when the soil sur-

face was sealed with paraffin. The stimulation of lower buds into active development was observed on stems defoliated by gas passed into the soil about the roots.

4. Two of the twenty trees that were gassed died before the beginning of the next growing season.

5. The trees in which the roots were exposed to 5 or 10 cu. ft. of gas in the soil exhibited no injurious effects the second season. Trees in soil gassed with 46.2 to 864.0 cu. ft. of gas exhibited signs of injury the second season. The injury consisted primarily of dried distal portions of some stems or branches. Below, the killed distal portions of stems or branches new, healthy, foliaged shoots developed. Very little definite injury to the roots of these gassed trees was observed in the second season.

6. It was not possible to assign limits for the volume of gas nor the period over which it must flow into the soil to produce definite degrees of injury or death.

7. From the similarity of these results to those obtained by using a "mixed" illuminating gas, and also ethylene and air mixtures enclosed about the roots of woody plants, it is suggested that ethylene or constituents of the coke oven gas with a physiological action similar to ethylene would explain the injuries, lethal, and stimulative effects upon these elm trees when roots were exposed to this coke oven gas in the soil.

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# GLUTAMINE METABOLISM OF THE BEET[1]

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## Introduction

Although glutamine has long been known as a plant constituent (**10**), the relationship of this amide to the nitrogen metabolism of the plant has seldom been subjected to direct test. The close analogy in chemical structure with asparagine and the similarity of the conditions under which the respective amides are found have led to the view, early expressed by SCHULZE (**6**), that glutamine replaces asparagine as the chief amide in many species and fulfills the same functions. This view is shared by PRIANISCHNIKOW (**4**).

SCHULZE regarded the asparagine, which accumulated in the etiolated seedlings of many species, as a secondary product of protein digestion (**7, 8, 9**), ammonia being the intermediate product. This idea was supported by the experiments of SUZUKI (**11**), who had found that young plants cultured upon solutions that supply nitrogen in the form of ammonium salts greatly increase their store of asparagine. PRIANISCHNIKOW (**5**) has shown that asparagine is synthesized in seedlings in response to the advent of ammonia derived from the decomposition of the protein of the cotyledons, the synthesis occurring both in light and in dark in the presence of carbohydrates, but only in light in their absence. He regards the reaction as a provision against the toxic effect of ammonia, the neutral amide being stored in readiness for subsequent syntheses in which nitrogen in readily available form is required. MOTHES (**3**) has found that asparagine fulfills a similar function in mature plants, and the experiments of VICKERY, PUCHER, WAKEMAN, and LEAVENWORTH (**15**) have also shown that amide nitrogen rapidly accumulates in the leaf of the tobacco plant when cultured in water in the dark. In this case the definite correlation that was observed between the disappearance of the amino nitrogen of the protein that underwent digestion and the increase in amide nitrogen strongly suggests that ammonia was an intermediate compound in the reaction, and makes it clear that the amino acids derived from the protein were promptly deaminized.

The writers have recently (**12**) observed that the tomato plant, when cultured upon solutions that provide ammonia as the sole source of nitrogen, responds by synthesizing glutamine in quantities considerably greater than those found in similar plants cultured upon nitrate. In this species, aspara-

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gine appears to play an entirely minor rôle as a detoxifying agent, and the observation clearly indicates that glutamine may serve in a mature plant as a detoxifying agent for ammonia of extracellular origin. The glutamine was found, in this species, to be stored chiefly in the stem, and the question was raised as to the location in the plant in which the synthesis of the glutamine actually occurred. Unfortunately the roots of the tomato plants studied were not analyzed.

The observations led us to believe, however, that the glutamine content of other species might be increased by treatment of the growing plants with ammonium salts, and in view of the importance of the root of the beet as a readily available source of glutamine (**13**), we have studied the effect of heavy dressings of ammonium sulphate applied to the growing plants.

Experiments of two types were conducted. The field experiment was designed to produce suitable material for large scale preparations of the amide; the greenhouse experiment was carried out with the object of forcing the plant to the limit of its capacity to synthesize glutamine.

## Field experiment

A 2 M solution of ammonium sulphate was applied to the soil in the field experiment on October 3, 1934, at the rate of 1500 pounds of nitrogen per acre to a crop of well grown beets (variety not known) that had been planted about July 1, following a crop of early spinach, on land fertilized the previous fall with pig manure and lime. A control sample of untreated plants was taken at the same time. On October 5, the treated plants were sampled, and an additional treatment of 500 pounds per acre of nitrogen as ammonium sulphate was applied to the remaining plants which were sampled on October 11. The samples consisted of approximately 6 kilograms of trimmed and washed roots. Glutamine was prepared from them according to the modifications of the method of SCHULZE and BOSSHARD which the writers have recently described (**13**), analyses for glutamine and asparagine being made by the indirect method (**14**) at each step in the procedure.

Table I shows that the increase in soluble nitrogen of the root tissue is almost exactly equivalent to the increase in glutamine nitrogen, that is to say, the nitrogen absorbed subsequent to the ammonium sulphate treatment was, in almost equal quantities, assimilated as glutamine. Table II shows that the immediate effect of the first treatment of the soil was to increase the free ammonia nitrogen content of the tissue about sevenfold, and to double the quantity of asparagine amide nitrogen. Nevertheless six days later, and regardless of a second heavy application of ammonium sulphate, the free ammonia decreased to less than half its maximum value and the asparagine amide nitrogen fell to its former low level. Meanwhile the glutamine continued to increase. Furthermore, although the asparagine increased slightly

TABLE I

INCREASE IN GLUTAMINE CONTENT OF BEET ROOTS BY APPLICATION OF HEAVY DRESSINGS OF AMMONIUM SULPHATE

| ROOT CONTENTS | QUANTITY OF CONTENTS* | | |
|---|---|---|---|
| | OCT. 2 | OCT. 5 | OCT. 11 |
| Soluble N | 1.958 | 2.104 | 2.164 |
| Glutamine in extract | 1.937 | 2.793 | 3.009 |
| Glutamine N in extract | 0.371 | 0.536 | 0.577 |
| Increase soluble N | ..... | 0.146 | 0.206 |
| Increase glutamine N | ..... | 0.164 | 0.205 |
| Glutamine N as percentage soluble N | 18.9 | 25.5 | 26.7 |
| Recrystallized glutamine isolated | 1.58 | 2.29 | 2.55 |

* Figures not otherwise designated are grams per kilogram of fresh tissue.

at first, the subsequent regression indicates that asparagine enters into the metabolism as a source of nitrogen for further syntheses, notwithstanding the entrance of large amounts of ammonia from without and the stress upon the plant of dealing with this ammonia by converting it to glutamine. In this species, the two amides clearly occupy different positions in the general scheme of nitrogen metabolism.

TABLE II

EFFECT UPON THE AMMONIA, ASPARAGINE AMIDE, AND GLUTAMINE AMIDE NITROGEN OF BEET ROOTS OF APPLICATION OF HEAVY DRESSINGS OF AMMONIUM SULPHATE

| ROOT CONTENTS | QUANTITY OF CONTENTS* | | |
|---|---|---|---|
| | OCT. 2 | OCT. 5 | OCT. 11 |
| | *gm.* | *gm.* | *gm.* |
| Ammonia N | 0.0035 | 0.025 | 0.010 |
| Asparagine amide N | 0.027 | 0.058 | 0.026 |
| Glutamine amide N | 0.186 | 0.268 | 0.289 |

* Figures are grams per kilogram of fresh tissue.

## Greenhouse experiment

The soil used in the greenhouse experiment was prepared with a dressing of 4–8–7 commercial fertilizer at the rate of 2000 pounds per acre and precipitated chalk was added at the rate of 3500 pounds per acre. Seeds of the Detroit Dark Red variety of beets were planted thickly June 15, 1934, in rows 9 inches apart. The plants were later thinned to permit proper development. The treatments with ammonium sulphate solution were begun on Sep-

tember 20. The salt was applied in 0.5 M solution at the rate of 150 pounds of nitrogen per acre daily, and applications were continued for 17 days or until October 8. At this time pronounced evidence of toxicity had developed and the plants were chlorotic and wilted. Samples of twenty plants were taken on the dates shown in table III and were separated into roots and tops. Ten plants of each sample, the roots of which had been cut into slices 1 to 2 mm. thick, were dried at 80° C. in an oven with rapid circulation of air. The other ten plants of each sample were extracted with cold water after ether cytolysis approximately as described by VICKERY, PUCHER, and CLARK (**13**). Analyses were made of both the water extracts and the dried tissue. The data are shown in table III. The ammonia values are derived from analyses of the water extracts. They are the more trustworthy because of the difficulty of drying fleshy tissue rapidly enough to avoid significant hydrolysis of glutamine. These ammonia values are used in the calculation of the amide values derived from the analysis of the dried samples.

The general effect of the ammonia treatment upon the plants is roughly shown by the weights of the roots and tops. Little or no growth occurred, and definite injury began to appear by October 4. The sample of October 8 consisted of plants with wilted leaves, some of which were quite yellow. A few of the leaves that had decomposed were discarded.

The plants of this series were all definitely richer in glutamine than the plants of the field experiment, but the response to the treatment with ammonia was even more striking. Free ammonia did not accumulate in the roots until a week had elapsed, and the asparagine showed no significant variations until the end of the treatment. Meanwhile the glutamine content increased rapidly. The quantity in the last sample (October 8) was twice that in the control samples of October 10 and 17.

Toward the end of the period of treatment, the free ammonia increased markedly in the roots, and the asparagine finally showed a significant increase, but this occurred only after definite, toxic effects became evident. Asparagine seems to have little part in the protection of the beet against ammonia intoxication while the plants remain in a healthy condition.

The effect of the ammonia treatment on the leaves and petioles of the beet is in striking contrast to the effect upon the roots. The free ammonia increased slowly during the first eight days, but a toxic level was not reached until later. The asparagine remained constant throughout, within the limits of experimental error, and the glutamine showed no significant increase until the final period of serious damage. It is clear that the protective mechanism in the beet against an influx of ammonia from the soil is a function of the root rather than of the leaf tissue. This may be allied with the fact that the roots of the beet provide an abundant store of carbohydrate. It seems probable that the accumulation of glutamine in the tops at the last stage is a sec-

## TABLE III

EFFECT OF DAILY APPLICATIONS OF AMMONIUM SULPHATE, AT THE RATE OF 150 POUNDS OF NITROGEN PER ACRE, ON THE AMMONIA, ASPARAGINE AMIDE, AND GLUTAMINE AMIDE NITROGEN OF BEET PLANTS

| Date | Time | Nitrogen | Roots* | | | | | Tops* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fresh weight (10 plants) | Reaction of extract | Ammonia N | Asparagine amide N | Glutamine amide N | Fresh weight (10 plants) | Reaction of extract | Ammonia N | Asparagine amide N | Glutamine amide N |
| | *days* | *Pounds per acre* | *gms* | *pH* | | | | *gm.* | *pH* | | | |
| Sept. 20 ... | 0 | 0 | 91.9 | 6.15 | 0.0114 | 0.0512 | 0.276 | 236 | 6.08 | 0.0146 | 0.0214 | 0.0446 |
| Sept. 21 ... | 1 | 150 | 82.5 | 5.75 | 0.0118 | 0.0740 | 0.359 | 255 | 5.95 | 0.0129 | 0.0100 | 0.0418 |
| Sept. 24 ... | 4 | 600 | 124.3 | ..... | 0.0139 | 0.0547 | 0.390 | 271 | ..... | 0.0195 | 0.0215 | 0.0400 |
| Sept. 28 ... | 8 | 1200 | 98.4 | 6.08 | 0.0254 | 0.0599 | 0.510 | 259 | 5.39 | 0.0250 | 0.0241 | 0.0604 |
| Oct. 8 ... | 18 | 2550 | 75.8 | 5.78 | 0.0864 | 0.129 | 0.816 | 129 | 5.56 | 0.0523 | 0.0200 | 0.1540 |
| Oct. 10 ... | 20 | 0 | 134.8 | 6.08 | 0.0078 | 0.0713 | 0.418 | 177 | 5.70 | 0.0132 | 0.0248 | 0.0584 |
| Oct. 17 ... | 27 | 0 | 211.2 | ..... | 0.0107 | 0.0416 | 0.388 | 265 | ..... | 0.0123 | 0.0177 | 0.0358 |

* Figures not otherwise designated are grams per kilogram of fresh tissue.

ondary result of damage to the root system, since the ammonia represented seems to have originated from intracellular reactions rather than by translocation from the roots.

The relative concentration of glutamine in the roots of these plants at the final stage of the experiment may perhaps be more vividly expressed in terms of dry matter of the tissue. The roots contained 15.8 per cent. of dry matter, and the figure 0.816 gm. of glutamine amide nitrogen per kilo of fresh tissue therefore represents 5.4 per cent. of glutamine in the dry matter of the root. This is considerably higher than the proportion (2 per cent.) found in the beets treated in the field. Whether or not it represents the limit to which the species may be driven is a matter for future experimentation with different varieties, but in any case it is clear that the capacity of this plant to protect itself against ammonia by the synthesis of glutamine is phenomenal.

## Discussion

The amide metabolism of plants is closely associated with the presence of, or availability of, ammonia in the tissues. This in turn is obviously determined, in certain cases, by the conditions in the soil. Thus it would be incorrect to assume that a given proportion of amide nitrogen, whether high or low, is characteristic of a given species. Amides are highly reactive metabolites and the quantity present at any time is largely dependent on the circumstances under which the plant has grown. CLARK's experiments on tomatoes (**1**) have shown that this species, like the beet, is able to synthesize large amounts of glutamine when ammonia is supplied, and under nitrate fertilization the amide content of tomato tissue is not exceedingly high.

SCHULZE's and PRIANISCHNIKOW's idea that asparagine and glutamine fulfill similar functions with respect to ammonia detoxification appears to be correct in general, but an amplification seems necessary in view of the results of the present experiments. In a given species only one of the amides fulfills this function with respect to an influx of ammonia so long as the plants remain normal. The other amide enters in this rôle in significant quantities only when the stress upon the plant has become acute. This appears to be the case with respect to the function of glutamine in the beet. On the other hand, the experiments of SUZUKI, the more recent ones of PRIANISCHNIKOW on seedlings, and particularly the work of MOTHES (**3**) on the leaves of the bean apparently furnish examples of the converse case in which asparagine fulfills the primary ammonia-detoxifying function. The whole problem is still unsolved, however. The function of the second amide is by no means clear, and very few experiments have been recorded in which the analytical methods, to say nothing of the methods employed for preparing the tissues for analysis, are above criticism.

A further statement regarding the function of the amide is necessary. It has been found that the increase in soluble nitrogen of beet root tissue, on treatment of the soil with ammonia salts, is equivalent to the glutamine nitrogen rather than to the glutamine amide nitrogen. Although the close quantitative agreement in the present experiment is probably fortuitous, it is apparent that both nitrogen atoms of glutamine function in the ammonia-detoxifying action. It is not difficult to account for the behavior of the amide group in this respect. A simple dehydration of the ammonium salt of glutamic acid leads to the amide, and KREBS (**2**) has recently shown that this reaction readily occurs in the kidney, brain cortex, and retina of a number of animals when glutamic acid and ammonia are supplied. But the fact that the $\alpha$ amino group also shares in the reaction is not so simply explained. It indicates that the precursor of the glutamine is non-nitrogenous, and suggests that the synthesis takes place in two distinct phases—a primary phase possibly allied in mechanism with the synthesis of other amino acids, and a secondary phase in which the ammonium salt of glutamic acid is converted to the amide. Speculation on the nature of the precursor is premature until more definite information regarding it is at hand.

## Summary

1. Beets grown either in the open field or in a greenhouse were treated with liberal amounts of ammonium sulphate solution applied to the soil. Under these circumstances the glutamine content of the root tissue rapidly increased, although the asparagine content was not affected. Daily applications were most effective in increasing the glutamine content. At the final stage, where definite toxicity supervened, a glutamine concentration of 5.4 per cent. of the dry weight of the root tissue was attained. There was little effect upon the composition of the tops. Only after severe damage had occurred did the concentration of glutamine in the tops increase.

2. In the beet plant, glutamine occupies the position of a primary detoxifying agent for ammonia. The function of the asparagine in the general metabolism is not clear, although this substance also increases slightly during the last stages of ammonia intoxification.

3. Both nitrogen atoms of glutamine share in the reaction, and the inference is obvious that the precursor of the glutamine is non-nitrogenous. It is suggested that the reaction proceeds in two stages: a primary formation of glutamic acid, perhaps by reactions analogous to the synthesis of other amino acids; and a subsequent dehydration of the ammonium salt of glutamic acid to the amide.

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# HEMICELLULOSE CONSTITUENTS IN ALFALFA ROOTS[1]

BERNARD A. BURKHART

## Introduction

The organic food reserves in the root of alfalfa (*Medicago sativa*) have been studied by various American investigators, with the object of correlating the findings with sound cultural practices (**3, 6, 22**). In these studies significant fluctuations in the hemicellulose content, as determined by a dilute acid hydrolysis, were noted. The purpose of this investigation was to subject the hemicellulose constituents of the alfalfa root to a more exact chemical study and thereby compare them to the related polysaccharide components of other plants (**5, 11, 23**).

Recently English and German investigators have studied this class of substances in some detail, with the result that our knowledge of them has been greatly extended and clarified. Noteworthy advances have been made by O'DWYER (**13–17**), NORRIS and PREECE (**12**), PREECE (**18, 19**), and by SCHMIDT (**20**), and LÜDTKE (**8–10**). Particularly valuable are the methods of fractionation and separation introduced by O'DWYER, and NORRIS and PREECE. HAWLEY and NORMAN'S (**4**) efforts to effect a rational system of nomenclature and classification of this complex group of polysaccharides have been a great aid, while LÜDTKE'S[2] (**8–10**) studies on the occurrence of these materials in the cell wall and their rôle and function in cell wall formation and metabolism are outstanding.

The hemicelluloses as a class are characterized by their ease of hydrolysis by dilute mineral acids and their solubility in dilute alkali. It is by extraction with alkali and subsequent precipitation that they are usually isolated. The properties of the hemicelluloses prepared by a dilute alkaline extraction are determined to a large extent by the concentration of alkali used, the conditions maintained during the extraction, and by the technique used to precipitate and purify them.

Perhaps the most significant factor is the concentration of alkali used and the temperature at which the extraction is conducted. Whether the action of hot dilute alkali has a detrimental effect is determined to a large extent by the original properties and composition of the hemicelluloses in question. It is possible that even very dilute alkali can effect an appreciable

[1] Research supported in part by grants from the graduate Research Committee. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] The papers by LÜDTKE discuss this field at some length and give extensive citations to the work of HÖAGLUND, SCHMIDT and coworkers, etc., published in Cellulosechemie during the interval 1927–34.

degradation of the macro molecules and thereby sever stable bonds with the production of free carbonyl groups. In those instances where polyuronides are present, hot dilute alkali might bring about decarboxylation (**1**) with the result that the final properties of the isolated preparation might be far afield from the original. Consequently all extractions in this investigation were conducted with dilute alkali at room temperature. Obviously the removal of a given hemicellulose fraction might not be complete, especially when the more dilute concentrations are employed (0.5–1.0 per cent.), but the tendency toward drastic change would be reduced. On the other hand, an incomplete extraction with 1 per cent. alkali might result in the contamination of fractions subsequently removed with more concentrated alkali. Consequently 5 per cent. sodium hydroxide was chosen as the extracting medium. In the light of the aforementioned limitations, what is presented here must be looked upon as a preliminary survey of the hemicellulose constituents of the alfalfa root. A more detailed study will most likely demand certain revisions of technique, and further partitioning of the fractions isolated; but it is thought that through this work a general insight into the character, chemical composition, and properties of the chief hemicellulose constituents of the alfalfa root has been gained.

## Experimentation

The technique employed for the extraction and fractionation of the hemicellulose constituents was essentially the same as that introduced by O'Dwyer (**13–17**), Norris and Preece (**12**) and Preece (**18, 19**). The methods employed by these investigators appeared to be the most appropriate at the time this work was started. Certain deviations from their technique conditioned by the specific nature of the root material at hand were obviously necessary. Only the principal deviations will be stated since in any study on the hemicelluloses the specific details employed hold only for the material in question.

Grimm alfalfa roots (*Medicago sativa*) were used in this study. The roots were dug in the early part of November 1929 and 1931, when the plants were in complete dormancy. They were cleaned, separated into the xylem and phloem tissue, and preserved in 80 per cent. ethanol.

### Preliminary extractions to remove fats, lipoids, sugars, pigments, and proteins

The ground material was extracted with 80 per cent. ethanol to remove soluble sugars and then extracted 24 hours with a mixture of 2 parts benzene and 1 part ethanol in a large Soxhlet extractor. The airdried material was then extracted with 1 per cent. ammonium hydroxide and washed free of ammonia with 55 per cent. ethanol.

### Extraction of hemicellulose with 5 per cent. sodium hydroxide

The hemicelluloses were extracted with 5 per cent. sodium hydroxide at room temperature with the aid of a shaking machine. The residue was removed by centrifugation and the solution clarified by passing it through a Sharples supercentrifuge.

### Precipitation and purification of hemicelluloses A and B extracted with 5 per cent. sodium hydroxide

The clarified extract was precipitated with an equal volume of 95 per cent. ethanol and the hemicellulose filtered off. The hemicellulose was redissolved in 5 per cent. alkali and reprecipitated with ethanol. After again dissolving in alkali the solution was made acid with hydrochloric acid and the precipitated hemicellulose filtered off, washed, and dried with ethanol and ether. After drying in a desiccator, the product was a fine, light, white, tasteless powder. This was designated hemicellulose A.

The acid filtrate from the above was mixed with an equal volume of 95 per cent. ethanol and a second hemicellulose thus obtained. This was similar in appearance to the first and was designated hemicellulose B.

### Fractionation of hemicelluloses A and B through use of Fehling's solution

These two hemicelluloses were now fractionated according to the procedure of Preece (**18**), using Fehling's solution. By this means hemicellulose A was fractionated into two portions designated hemicelluloses A-1 and A-2. Hemicellulose A-1 forms an insoluble copper complex upon the addition of Fehling's solution to an alkaline solution of the hemicellulose. Hemicellulose A-2 was obtained from the filtrate from this precipitation by making the solution acidic with hydrochloric acid and by adding three volumes of 95 per cent. ethanol.

Hemicellulose B was treated in the same manner and two fractions, hemicelluloses B-1 and B-2, were obtained. The copper complex of hemicellulose B-1 is not insoluble and must be precipitated by the addition of one-fourth volume of 95 per cent. ethanol after mixing the Fehling's solution and the alkaline hemicellulose solution. Hemicellulose B-2 is obtained in the same manner as hemicellulose A-2. Both hemicelluloses B-1 and B-2 are soluble in water and consequently must be washed with dilute ethanol to remove salts and acid. Hemicellulose A-2 was obtained in approximately one-half the quantity of hemicellulose A-1. The quantity of hemicellulose B-2 obtained was very small.

### Properties of hemicelluloses extracted with 5 per cent. sodium hydroxide

Hemicellulose A is extracted with 5 per cent. sodium hydroxide. It is precipitated by acidifying the alkaline extract and is insoluble in water and solvents. It forms a water insoluble copper complex upon addition of Fehling's solution to an alkaline solution of the hemicellulose. A fraction of hemicellulose A does not form an insoluble copper complex and may be separated from the main portion by this means.

Hemicellulose B is extracted with 5 per cent. sodium hydroxide. It is not precipitated upon acidification of the extract but is thrown down by the addition of ethanol. It forms a water soluble copper complex with Fehling's solution which may be precipitated by the addition of one-fourth volume of ethanol. A small portion is not precipitated and may be separated as a separate fraction.

The general scheme employed for the separation and purification of the hemicelluloses of the alfalfa root is indicated on page 425.

### Quantitative analysis of hemicelluloses extracted with 5 per cent. sodium hydroxide

The hemicellulose preparations were dried in a vacuum oven at 70° and 15 mm. pressure for 30 hours. The analytical results were then calculated on an absolute dry basis. The ash analyses were made according to the Pregl micro technique.

Reducing power.—Samples were hydrolyzed in 2.5 per cent. sulphuric acid for 3 hours. The sulphuric acid was removed quantitatively and the reducing power determined by the method of Shaffer and Hartmann (**21**).

Uronic acid content.—The uronic acid content was determined by decarboxylation with 12 per cent. hydrochloric acid according to the method recently developed in this laboratory (**2**).

Pentosan content.—The pentosan content of the samples was determined by distilling with 12 per cent. hydrochloric acid after the Kröber-Tollens method (**7**). The pentosan determinations were all corrected for the error introduced by the furfural arising from the uronic acids present.[3]

Specific rotation.—The rotations were read on 0.25 gm. samples dissolved in 2.5 per cent. sodium hydroxide and made up to 25.0 cc. The readings were made on a Schmidt and Haensch saccharimeter and calculated to specific rotation.

The analytical data for the various hemicelluloses before and after the Fehling's solution purification are given in tables I and II.

[3] The correction made is only an approximation (see Norris, F. W., and Resch, C. E. The analysis of carbohydrates in the cell wall of plants. I. The relation between uronic anhydride content and furfuraldehyde yield. Biochem. Jour. 29: 1590–1596. 1935).

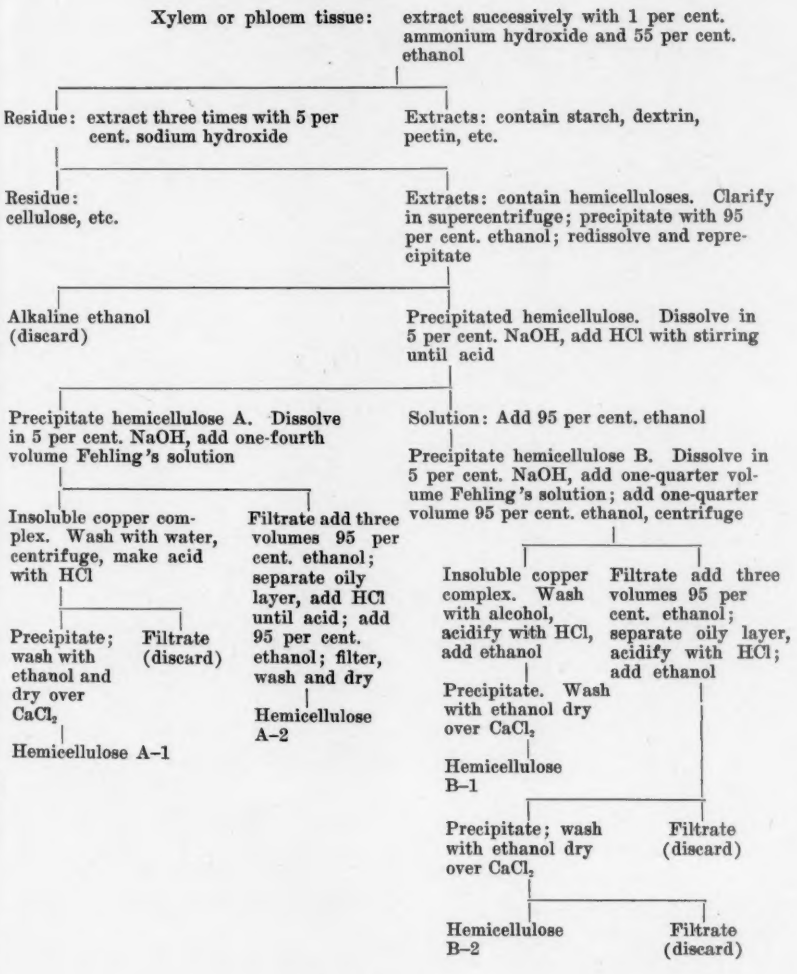
Xylem or phloem tissue: extract successively with 1 per cent. ammonium hydroxide and 55 per cent. ethanol
Residue: extract three times with 5 per cent. sodium hydroxide
Extracts: contain starch, dextrin, pectin, etc.
Residue: cellulose, etc.
Extracts: contain hemicelluloses. Clarify in supercentrifuge; precipitate with 95 per cent. ethanol; redissolve and reprecipitate
Alkaline ethanol (discard)
Precipitated hemicellulose. Dissolve in 5 per cent. NaOH, add HCl with stirring until acid
Precipitate hemicellulose A. Dissolve in 5 per cent. NaOH, add one-fourth volume Fehling's solution
Solution: Add 95 per cent. ethanol
Precipitate hemicellulose B. Dissolve in 5 per cent. NaOH, add one-quarter volume Fehling's solution; add one-quarter volume 95 per cent. ethanol, centrifuge
Insoluble copper complex. Wash with water, centrifuge, make acid with HCl
Filtrate add three volumes 95 per cent. ethanol; separate oily layer, add HCl until acid; add 95 per cent. ethanol; filter, wash and dry
Insoluble copper complex. Wash with alcohol, acidify with HCl, add ethanol
Filtrate add three volumes 95 per cent. ethanol; separate oily layer, acidify with HCl; add ethanol
Precipitate; wash with ethanol and dry over CaCl2
Filtrate (discard)
Hemicellulose A–2
Precipitate. Wash with ethanol dry over CaCl2
Hemicellulose A–1
Hemicellulose B–1
Precipitate; wash with ethanol dry over CaCl2
Filtrate (discard)
Hemicellulose B–2
Filtrate (discard)

TABLE I

COMPARATIVE ANALYSIS OF HEMICELLULOSES OF XYLEM AND PHLOEM TISSUE EXTRACTED WITH 5 PER CENT. SODIUM HYDROXIDE

| SAMPLE | ASH | HEXURONIC ACID | PENTOSE | REDUCING POWER | SPECIFIC ROTATION |
|---|---|---|---|---|---|
| Xylem Hemicellulose A ..... | 7.27 | 6.51 | 28.4 | 87.1 | − 54.5° |
| Xylem Hemicellulose B ..... | 1.57 | 1.85 | 12.7 | 76.0 | −126.9° |
| Phloem Hemicellulose A ..... | 6.0 | 5.15 | 26.8 | 83.5 | − 61.5° |
| Phloem Hemicellulose B ..... | 1.41 | 8.60 | 2.78 | 65.5 | −141.2° |

TABLE II

COMPOSITION OF HEMICELLULOSE OF XYLEM TISSUE AFTER FRACTIONATION THROUGH USE OF FEHLING'S SOLUTION

| SAMPLE | ASH | URONIC* ACID | PENTOSE | REDUCING POWER | SPECIFIC ROTATION |
|---|---|---|---|---|---|
| Xylem Hemicellulose A–1 | 0.93 | ..... | 85.0 | 86.7 | −51.3° |
| Xylem Hemicellulose B–1 | 0.44 | ..... | 0.02 | 55.5 | −84.1° |

* The quantity of hexuronic acid was too small to yield accurate results.

## Summary

1. The hemicelluloses of the phloem and xylem tissue of alfalfa roots soluble in 5 per cent. sodium hydroxide have been isolated, purified, and analyzed.

2. The hemicelluloses extracted with 5 per cent. sodium hydroxide were partitioned into two fractions, an acid insoluble fraction (hemicellulose A) and an acid soluble alcohol insoluble fraction (hemicellulose B). Through the use of Fehling's solution two additional fractions were obtained, hemicelluloses A-2 and B-2.

3. The chief sugars found in all fractions were the pentose d-xylose and the hexose d-glucose. Some hexuronic acid (d-glucuronic acid) is also present.

4. The most significant variation between the hemicelluloses of the phloem and xylem tissue is in the xylose content, which is highest in the

xylem hemicelluloses. On the other hand, the content of d-glucuronic acid is highest in the phloem hemicellulose.

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# REDUCTION OF NITRATES TO NITRITES BY THE EXPRESSED JUICE OF HIGHER GREEN PLANTS

ANNA L. SOMMER

## Introduction

It is believed by some that there are substances present in green plants that catalytically reduce nitrates to nitrites in the absence of light. Such reduction was reported by ECKERSON (**2, 3, 4, 5**). The reduction appeared to be catalytic and occurred in the presence or absence of light. Oxygen seemed to be necessary for the reaction. On the other hand, no catalytic reduction was found by ANDERSON (**1**) in his work with expressed plant juices. ECKERSON obtained less reduction with the juice of plants grown for a time in a phosphorus-deficient medium than in a medium with adequate phosphate. In a study of the functions of phosphate in plants, this phase of ECKERSON's work was viewed with special interest and experiments were set up to determine whether phosphorus aided in the formation of compounds causing this reduction or whether the presence of phosphate was necessary for the reaction. The problem, however, soon resolved itself into that of determining whether or not substances causing catalytic reduction of nitrate to nitrite really existed in the green plant. The results reported in this paper deal principally with the latter question.

## Experimentation

Tomato plants were used in most of the work since they were used in much of the work reported by ECKERSON. Cotton, wheat, buckwheat, and narcissus plants also were used. Determinations were made of the reducing action of the expressed juice of both the tops and the roots. The method used in the first experiments was that described by ECKERSON (**3**):

> "1 cc. plant juice, 1 cc. 10% $KNO_3$ and approximately 10 mg. glucose were made up to 10 cc., put in a 50-cc. Erlenmeyer flask and brought to pH 7.2 to 7.4 with NaOH, using phenol red as indicator. Toluene (7 to 10 drops) was then added, the flask shaken, and put in an oven at 35° C."

The nitrite was determined colorimetrically by means of the sulphanilic reagent also described by ECKERSON (**3**). In certain series the flasks were placed in a waterbath instead of an oven. There also may have been another difference in technique, since the flasks were stoppered (no mention was made in the preceding directions of stoppering the flasks).

Three methods of preparing the material for expressing the juice were

tested: (1) freezing, (2) chopping fine, and (3) grinding with sand. The juice was extracted by pressing the material between several thicknesses of cheesecloth. The results showed that the method of preparation did not affect the results; consequently in later experiments all samples were ground with sand.

When the flasks were tightly stoppered with rubber stoppers there was never more than a very small amount and often no nitrite formed. When the flasks were loosely stoppered variable results were obtained, and when considerable amounts of nitrite were formed duplicates seldom checked. This was particularly noticeable in cases where the flasks were placed in a waterbath instead of an oven. The absence of the odor of toluene in flasks where much nitrite was present suggested contamination by microorganisms. The following variations in the procedure were then made in an effort to explain these discrepancies:

1. Flasks tightly stoppered with well fitting rubber stoppers (check).
2. Flasks left unstoppered.
3. Expressed juice evaporated to dryness at 100° C.
4. Expressed juice evaporated to dryness at 110° C.
5. 300-cc. instead of 50-cc. flasks (stoppered with well fitting rubber stoppers).
6. Nitrite determinations made at different times (1 to 24 hours) on the same samples.
7. Five to six drops of toluene added when the juice, nitrate, and glucose were mixed; five to six drops added at the end of 6 hours; and five to six drops added at the end of 12 hours, after which the flasks were stoppered. These were compared with flasks to which ten drops of toluene were added immediately, left unstoppered for 12 hours and then stoppered. Nitrite determinations were made at the end of 24 hours.
8. Samples in two 250-cc. flasks in series. Ten drops of toluene were added to each flask. In one procedure, *a*, toluene-saturated air was drawn through the samples and in another, *b*, it was drawn over the surface, Variations 8*a* and 8*b* were used only once, while all others were repeated many times. The amount of nitrite formed was compared each time with that in the check. In the nitrite determinations the samples were centrifuged after the addition of the reagent. This greatly facilitated the comparison with the standard. Representative data are shown in tables I and II.

Oxygen did not seem to be a limiting factor since no more nitrite was formed in 300-cc. flasks than in 50-cc. flasks. Moreover, there was no more nitrite formed where air was drawn through the flasks than in the checks.

Evidence that a high concentration of toluene was not an inhibiting factor, other than in preventing the growth of nitrite-forming microorganisms, was furnished by the results of experiments in unstoppered flasks to

TABLE I

REDUCTION UNDER VARIOUS CONDITIONS OF NITRATE TO NITRITE BY EXPRESSED JUICE OF TOMATO PLANTS (SAME AMOUNT OF TOLUENE

TABLE I

REDUCTION UNDER VARIOUS CONDITIONS OF NITRATE TO NITRITE BY EXPRESSED JUICE OF TOMATO PLANTS (SAME AMOUNT OF TOLUENE ADDED IN ALL CASES)

| Sample no. | Part of plant | Nitrite nitrogen formed in presence of 1 cc. expressed juice in oven at 35° C. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Flasks stoppered | | | | | Flasks unstoppered | | | |
| | | Unheated | | | Evaporated to dryness at 100° C. | | Unheated | | Evaporated to dryness at 100° C. | |
| | | 50-cc. | | 300-cc. | 50-cc. | | 50-cc. | | 50-cc. | |
| | | 3 hours | 20 hours | 20 hours | 3 hours | 20 hours | 3 hours | 20 hours | 3 hours | 20 hours |
| | | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 1 | Tops | ..... | Trace* | Trace | Trace | Trace | Trace | 0.05 | Trace | 0.08 |
| | | ..... | " | " | " | " | " | 0.0016 | " | 0.0041 |
| | Roots | ..... | ..... | " | ..... | " | " | 0.0056 | " | 0.125 |
| | | ..... | ..... | " | ..... | " | " | 0.0062 | " | 0.006 |
| 2 | Tops | ..... | Trace | " | ..... | " | ..... | Trace | " | Trace |
| | | ..... | " | " | ..... | " | ..... | " | " | " |
| | Roots | ..... | " | " | ..... | " | ..... | " | " | " |
| | | ..... | " | " | ..... | " | ..... | " | " | " |
| 3 | Tops | ..... | " | " | ..... | " | Trace | " | " | 0.0026 |
| | | ..... | " | " | ..... | " | " | 0.0012 | " | 0.200 |
| | Roots | ..... | 0.0002 | 0.0002 | ..... | 0.0002 | 0.0002 | 0.0002 | 0.0002 | 0.0002 |
| | | ..... | 0.0002 | 0.0002 | ..... | 0.0002 | 0.0002 | 0.0002 | 0.0002 | 0.0002 |
| 4 | Tops | ..... | ..... | None | ..... | ..... | ..... | 0.100 | ..... | ..... |
| | | ..... | ..... | " | ..... | ..... | ..... | 1.60 | ..... | ..... |
| | Roots | ..... | ..... | 0.0014 | ..... | ..... | ..... | 0.0018 | ..... | ..... |
| | | ..... | ..... | 0.0014 | ..... | ..... | ..... | 0.0124 | ..... | ..... |
| 5 | Tops | None | None | None | ..... | ..... | None | None | ..... | ..... |
| | | " | " | " | ..... | ..... | " | " | ..... | ..... |
| | Roots | " | " | " | ..... | ..... | " | 0.020 | ..... | ..... |
| | | " | " | " | ..... | ..... | " | 0.030 | ..... | ..... |
| | | Water bath 35° C. | | | | | | | | |
| 6 | Tops | None | None | None | None | None | None | None | None | 0.046 |
| | | " | " | " | " | " | " | 0.0022 | " | 0.124 |
| | Roots | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| | | " | " | " | " | " | " | " | " | 0.0078 |
| 7 | Tops | ..... | " | ..... | ..... | " | ..... | 0.46 | ..... | 0.08 |
| | | ..... | " | ..... | ..... | " | ..... | 0.016 | ..... | 0.26 |
| | Roots | ..... | " | ..... | ..... | " | ..... | 0.033 | ..... | 0.029 |
| | | ..... | " | ..... | ..... | " | ..... | Trace | ..... | 0.024 |

* Less than 0.0001.

## TABLE II

REDUCTION UNDER VARIOUS CONDITIONS OF NITRATE TO NITRITE BY EXPRESSED JUICE OF TOMATO PLANTS (DIFFERENT AMOUNTS OF TOLUENE ADDED)

| SAMPLE NO. | PART OF PLANT | NITRITE NITROGEN FORMED IN PRESENCE OF 1 CC. EXPRESSED JUICE | | | |
|---|---|---|---|---|---|
| | | FIFTY-CC. FLASKS STOPPERED 24 HOURS; TOLUENE 10 DROPS | 50-CC. FLASKS UNSTOPPERED 12 HOURS, STOPPERED 12 HOURS | | |
| | | | TOLUENE 15–18 DROPS | | TEN DROPS TOLUENE ADDED AT ONCE |
| | | | 5–6 DROPS ADDED AT ONCE, THE REST AT 6-HOUR INTERVALS | ALL ADDED AT ONCE | |
| | | *mg.* | *mg.* | *mg.* | *mg.* |
| 8 | Tops | None | None | ..... | None |
| | | " | " | ..... | " |
| | Roots | 0.0010 | 0.0010 | ..... | 0.042 |
| | | 0.0010 | 0.0010 | ..... | 0.100 |
| 9 | Tops | Trace* | Trace | ..... | 0.071 |
| | | " | " | ..... | 0.111 |
| | Roots | " | " | ..... | Trace |
| | | " | " | ..... | " |
| 10 | Tops | " | " | ..... | " |
| | | " | " | ..... | " |
| | Roots | " | " | ..... | " |
| | | " | " | ..... | " |
| 11 | Tops | " | " | 0.0044 | " |
| | | " | " | Trace | " |
| | Roots | " | " | 0.0018 | 0.084 |
| | | " | " | 0.0017 | 0.100 |
| 12 | Tops | " | " | ..... | Trace |
| | | " | " | ..... | 0.0062 |
| | Roots | " | " | ..... | 0.072 |
| | | " | " | ..... | 0.125 |

* Less than 0.0001.

which only five drops of toluene were added at the beginning, five drops at the end of 6 hours, and five drops at the end of 12 hours, after which the flasks were stoppered. There was no more nitrite produced in these than in flasks that had been stoppered from the beginning with ten drops of toluene, and sometimes less than in those to which ten drops of toluene had been added immediately on mixing and the flasks left unstoppered for the first 12 hours. Results of experiments under these conditions also furnished further evidence that oxygen was not a limiting factor.

Evidence that nitrate might be reduced by green plants in the dark was furnished by NIGHTINGALE and his coworkers (**7, 8, 9, 10**). They showed

## TABLE III

REDUCTION UNDER VARIOUS CONDITIONS OF NITRATE TO NITRITE* BY EXPRESSED JUICE OF ROOTS AND GROUND TISSUE OF BULBS AND LEAVES OF *NARCISSUS GRANDIFLORA*

| TIME OF GROWTH | NITRITE NITROGEN FORMED IN PRESENCE OF 1 CC. JUICE OR 1.5 GM. TISSUE | | | | | |
|---|---|---|---|---|---|---|
| | JUICE OR TISSUE UNHEATED 250-CC. FLASKS | | | EVAPORATED TO DRYNESS AT 110° C. 250-CC. FLASKS | | |
| | 2 HOURS | 5 HOURS | 20 HOURS | 2 HOURS | 5 HOURS | 20 HOURS |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| Bulbs and leaves (juice) 10 days | 0.0004<br>0.0004 | .....<br>..... | 0.0006<br>0.0008 | 0.0008<br>0.0008 | .....<br>..... | 0.0012<br>0.0012 |
| Roots 10 days | 0.0008<br>0.0008 | .....<br>..... | 0.0010<br>0.0010 | 0.0008<br>0.0008 | .....<br>..... | 0.0010<br>0.0010 |
| Bulbs and leaves (tissue) 12 days | .....<br>..... | .....<br>..... | 0.0016<br>0.0016 | .....<br>..... | .....<br>..... | .....<br>..... |
| Roots 12 days | .....<br>..... | .....<br>..... | 0.0016<br>0.0016 | .....<br>..... | .....<br>..... | .....<br>..... |
| Bulbs and leaves (tissue) 14 days | 0.0014<br>0.0012 | .....<br>..... | 0.0012<br>0.0010 | 0.0010<br>0.0012 | .....<br>..... | 0.0010<br>0.0012 |
| Roots 14 days | 0.0012<br>0.0012 | .....<br>..... | 0.0012<br>0.0010 | 0.0015<br>0.0015 | .....<br>..... | 0.0010<br>0.0013 |
| Bulbs and leaves (tissue) 8 days | 0.0004<br>0.0004 | 0.0010<br>0.0010 | 0.0010<br>0.0010 | 0.0008<br>0.0008 | 0.0012<br>0.0012 | 0.0011<br>0.0011 |
| Roots 8 days | 0.0016<br>0.0016 | 0.0020<br>0.0020 | 0.0020<br>0.0020 | 0.0020<br>0.0022 | 0.0026<br>0.0028 | 0.0026<br>0.0029 |
| Bulbs and leaves (tissue) 14 days – P | .....<br>..... | .....<br>..... | 0.0008<br>0.0008 | .....<br>..... | .....<br>..... | .....<br>0.0006 |
| Roots 14 days – P | .....<br>..... | .....<br>..... | 0.0010<br>0.0014 | .....<br>..... | .....<br>..... | 0.0010<br>0.0010 |
| Bulbs and leaves (tissue) 14 days + P | .....<br>..... | .....<br>..... | 0.0014<br>0.0010 | .....<br>..... | .....<br>..... | 0.0012<br>0.0016 |
| Roots 14 days + P | .....<br>..... | .....<br>..... | 0.0012<br>0.0012 | .....<br>..... | .....<br>..... | 0.0012<br>0.0012 |

* No trace of nitrite detected with plants harvested on 16th and 18th days.

gains in organic nitrogen when asparagus, narcissus, and apple trees were grown in the dark in media containing nitrate. Experiments with paper white narcissus grown in the dark were therefore included in this study. In the first experiment dormant bulbs were placed in sand moistened with a complete nutrient solution. The first lot of the bulbs was harvested at the end of 10 days and the last at the end of 14 days. The bulbs and leaves and the roots were ground separately, with a little sand. The juice of the roots was easily expressed, but so little from the bulbs and leaves could be obtained, even at high pressure, that 1.5 gm. of the finely ground tissue instead of 1 cc. of the juice was used in all cases except the first. In this case the juice was so foamy that measurement by pipette was inaccurate. Tightly stoppered 300-cc. flasks were used in all cases. Unheated aliquots and aliquots evaporated to dryness at 110° C. were used. Determinations were made at the end of 2, 5, and 20 hours.

Appreciable amounts (table III) of nitrite were obtained in both the heated and the unheated aliquots. A little more nitrite was obtained at the end of 5 and 20 hours than at the end of 2 hours. In one case there was appreciably more reducing action by aliquots that had been evaporated to dryness at 110° C. than by unheated aliquots. It was noted in this case that the light brown color developed on heating the juice of the roots disappeared almost entirely as soon as the nitrate was added.

In the second experiment the bulbs were placed in sand moistened in some cases with a complete nutrient solution and in others with a nutrient solution lacking phosphate. These plants were harvested 8 to 18 days later and the procedure of the first experiment followed, except that the determinations were made only at the end of 20 hours. There was considerable difference in the amount of growth of plants in cultures with and without phosphate, especially after the first week, but there was no appreciable difference in the amount of nitrite formed. No nitrite was found for samples of plants harvested after the second week. In one case aliquots of which the pH was adjusted to 4.8, 6.0, and 8.0 were included but no difference in the amount of nitrite was found.

## Discussion

The work reported here does not support the theory that nitrates may be reduced to nitrites catalytically in the dark by the green plant. The results of this work, dealing only with expressed plant juices, is in agreement with that of ANDERSON (**1**) but not with that of ECKERSON (**2, 3, 4, 5**). The small amount of reduction obtained by treating nitrates with the expressed juice of narcissus plants in the early stages of growth, as well as that sometimes obtained by the use of other plants, may be due to easily

oxidizable compounds which might not come in contact with the nitrates in the intact plant or by easily oxidizable compounds which may occur only at certain stages of growth as is suggested by the work of NIGHTINGALE (7), in which he found appreciable amounts of nitrite present in the roots of plants in the early stages of growth and only a trace or none later. On the other hand, it is not conclusive evidence against the theory, since conditions produced by this treatment of the material may be such as to prevent the formation of nitrite or to cause its oxidation or reduction as soon as it is formed. In much of the work indicating the utilization of nitrates by the green plant in the dark, sufficient precaution has not been taken to prevent the action of contaminating microorganisms. The work of MUENSCHER (6) appears to be a striking exception. He found that *Chlorella* in pure culture, when furnished a suitable carbohydrate, made good growth in the dark with nitrate as the only source of combined nitrogen. This ability to utilize nitrate in the absence of light, however, may be characteristic of some algae and not found in the higher green plants.

## Summary

A large number of nitrite determinations on mixtures of nitrate, glucose, and expressed plant juices to which toluene had been added and maintained at sufficient concentration to prevent the action of microorganisms failed to indicate the presence of substances in the green plant which cause catalytic reduction of nitrate to nitrite in the absence of light.

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# SIMPLE APPARATUS FOR THE QUANTITATIVE DETERMINATION OF PHOTOSYNTHETIC AND RESPIRATORY RATIOS[1]

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(WITH ONE FIGURE)

## Introduction

A relatively simple apparatus for determining respiratory and photosynthetic ratios quantitatively in a reasonably accurate manner is desirable equipment for every plant physiology laboratory. The apparatus to be described, while not considered a precision instrument, has been found serviceable for this purpose. While designed primarily for student use in the laboratory work of plant physiology courses, it might also prove satisfactory for research if not too great accuracy of results is required. The apparatus depends, in principle, upon the measurement of changes in the volume of the $CO_2$ gas present in the apparatus by absorption in a KOH solution, and upon the measurement of changes in a volume of the $O_2$ gas present by manometric means. Results of sufficient quantitative exactness to demonstrate clearly the important principles regarding photosynthetic and respiratory ratios can easily be obtained with this apparatus.

## Apparatus

Figure 1 illustrates the arrangement of the apparatus, which consists of the following essential parts:

*A*. Stationary 50-cc. burette equipped with a stopcock *H* at its upper end, and connected with a glass tube leading to the glass chamber *C* by a length of rubber tubing *L*.

*B*. Vertically adjustable 50-cc. burette. Burettes *A* and *B* are connected at their lower ends with a length of rubber tubing *K*.

*C*. Photosynthetic (or respiratory) chamber.

*D*. Separatory funnel provided with a stopcock *I*, and a side tube with a stopcock *E*.

*F*. Thermometer.

*G*. Three-way stopcock of the design indicated.

*J*. Heavy rubber stopper.

Details of the assembled apparatus are shown in figure 1. All of the parts are standard laboratory equipment except the modified separatory funnel, which must be made by a glass blower, and the photosynthetic chamber, which was improvised from a small glass tumbler about 11 cm.

[1] Papers from the Department of Botany, Ohio State University, no. 367.

high and 6 cm. in top inside diameter. The rubber tubing used should be of seamless red rubber in order to minimize loss of $CO_2$ gas by diffusion through the rubber. Impermeability of the rubber stopper to the gas can be assured by coating its lower surface with asphaltum which has been cut with turpentine. The glass parts can be of ordinary soft glass, although Pyrex glass would probably prove more serviceable in certain respects.

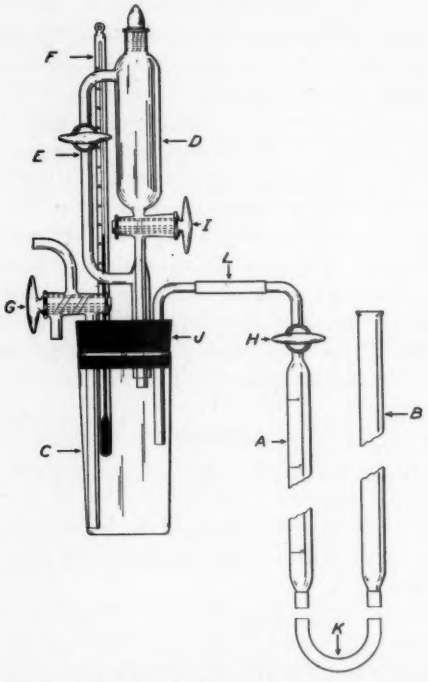


FIG. 1. Apparatus for the determination of photosynthetic and respiratory ratios. Description in the text.

It is essential that the system be airtight, since attempted measurements in the absence of leakproof connections will be worthless. The apparatus can be tested for leaks by filling it completely with water, adjusting burette *B* so that the level of the water in it will be considerably lower than in the photosynthetic chamber, and allowing the entire set-up to stand for several hours. If no change occurs in the level of the water in burette *B* during this period, leakage in the apparatus may be considered negligible.

### Procedure for determination of photosynthetic ratios[2]

The aggregate volume of the photosynthetic chamber *C*, separatory funnel *D*, including the side tube *E*, connecting tube *L*, and burette *A* to the zero mark on that burette, must first be determined. This may be done by measuring the volume of water required to fill this part of the apparatus, or by determining its increase in weight after filling it with water. This measurement should be made as accurately as possible and the determined volume recorded.

Burettes *A* and *B* should each be about half full of water. A leaf of some suitable species should now be introduced into the photosynthetic chamber. The petiole of the leaf should dip into a small vial of water. The volume of the leaf and the vial should first be determined by immersing in a graduated cylinder and noting the volume of water displaced. The total volume of leaf, vial, and water must be subtracted from the volume of the apparatus as determined above.

With the apparatus assembled, and stopcocks *E* and *I* closed, 30 cc. of a 25 per cent. KOH solution should now be poured into the separatory funnel *D*. The funnel should then be stoppered, stopcocks *E* and *H* opened, and burette *B* raised or lowered until water stands at the same level in both burettes. Stopcock *E* should then be closed. The volume of the photosynthetic chamber, after subtraction of the volume of the leaf, the vial, and the water should now be further corrected by subtracting the volume of the KOH solution. This final corrected volume will be designated as volume V.

The upper arm of stopcock *G* should now be connected to a simple type of gas-measuring burette which is in turn connected with a tank of pure $CO_2$ gas. The $CO_2$ should then be allowed to flow through the connecting rubber tube and out into the air through stopcock *G* until all of the other gases are swept out of the tube. Stopcock *G* should then be turned off, and the gas-measuring burette adjusted until it contains a known volume (usually approximately 25 cc.) of $CO_2$ gas[3] at atmospheric pressure. Stopcock

[2] Since respiration also is occurring in the green parts of a plant, the apparent volume of $CO_2$ consumed is less than the volume utilized in photosynthesis by the amount of the volume released in respiration, and similarly, the apparent volume of the $O_2$ released is less than the actual amount liberated in photosynthesis by the amount of volume of $O_2$ consumed in respiration. Hence the apparent photosynthetic ratio is a result of the differential effect between the two processes of photosynthesis and respiration. Since, in rapidly photosynthesizing leaves, the rate of photosynthesis is usually ten or more times as great as the rate of respiration, and the respiratory ratio of most leaves is usually almost unity, the simultaneous occurrence of respiration is not an appreciable source of error in determinations of the photosynthetic ratio in green leaves.

[3] The gas in commercially furnished tanks of $CO_2$ gas is not always 100 per cent. pure. For precise work it may be desirable to analyze the sample of gas used, and to introduce the necessary correction in determining the volume of gas which has been added. For demonstration purposes, however, this precaution can usually be neglected.

*G* should then be opened (stopcock *H* also being open), and the reservoir of the burette raised until the desired volume of $CO_2$ gas has been delivered into the system. As the gas is driven into the photosynthetic chamber burette *B* should be gradually lowered so that the menisci are maintained at the same level in both burettes during the transference of the $CO_2$ gas. Stopcock *G* should now be closed and, after allowing the apparatus to stand for a few minutes, any final adjustment of burette *B* necessary to equalize the level of the menisci in the two burettes should be made. Stopcock *H* should then be closed. The exact position of the water meniscus in burette *A* should now be recorded and the volume of the gas below the zero mark in the burette added to volume V. The sum of these two volumes will be designated as volume X.

The temperature of the photosynthetic chamber and the barometric pressure should also be recorded at the beginning of the determination.

The apparatus should be allowed to stand for six to eight hours in (preferably) a strong diffuse light. Any considerable heating of the gas mixture in the photosynthetic chamber above the temperature of the surrounding atmosphere should be avoided, since this makes it more difficult to secure satisfactory results. The intensity of the light can be reduced, if necessary, and the light diffused by shading the apparatus with one or more layers of cheesecloth.

At the end of this period stopcock *H* should be opened and the water menisci should be brought to the same level in both burettes by adjusting the position of burette *B*. Volume V plus the volume of gas below the zero mark in burette *A* at this reading will be designated as volume Y.

Stopcocks *E* and *I* should now be opened simultaneously and the KOH solution allowed to flow into the bottom of the photosynthetic chamber *C*. It should not be allowed to come in contact with the leaf. About 15 minutes should be allowed for the absorption of the $CO_2$ gas remaining in the apparatus. At the end of this period the water menisci should again be brought to the same level in the two burettes by adjusting the position of burette *B*. Volume V plus the volume of gas below the zero mark on burette *A* at this reading will be designated as volume Z.

The temperature of the photosynthetic chamber and the barometric pressure must be recorded again at the end of the experiment. All three of the recorded gas volumes (X, Y, and Z) must be reduced to standard conditions and corrected for aqueous tension. The aqueous tension of the gas within the apparatus may be assumed to correspond to that of a saturated atmosphere at the temperature of the apparatus.

The significance of the several measured volumes is as follows:

X = atmospheric gases + added $CO_2$
Y = atmospheric gases + unused $CO_2$ + $O_2$ released in photosynthesis
Z = atmospheric gases + $O_2$ released in photosynthesis

Hence, assuming A to represent the volume of $CO_2$ gas added (reduced to standard conditions):

$$Y - Z = CO_2 \text{ not consumed}$$
$$A - (Y - Z) = CO_2 \text{ consumed}$$
$$Z - (X - A) = O_2 \text{ released}$$
$$\frac{A - (Y - Z)}{Z - (X - A)} = \text{photosynthetic ratio}$$

Representative results obtained in the measurement of the photosynthetic ratios of the leaves of various species by means of this apparatus are given in table I.

TABLE I

PHOTOSYNTHETIC RATIOS

| SPECIES | $CO_2$ CONSUMED | $O_2$ RELEASED | PHOTOSYNTHETIC RATIO, $\frac{CO_2}{O_2}$ |
|---|---|---|---|
| | *cc.* | *cc.* | |
| Coleus | 14.6 | 14.9 | 0.979 |
| (*Coleus blumei*) | 10.2 | 10.4 | 0.980 |
| | 18.7 | 18.8 | 0.995 |
| Geranium | 52.8 | 52.8 | 1.000 |
| (*Pelargonium hortorum*) | 22.7 | 23.0 | 0.987 |
| Tulip tree | 12.6 | 12.5 | 1.008 |
| (*Liriodendron tulipifera*) | 11.6 | 11.9 | 0.974 |
| American elm | 13.9 | 13.1 | 1.061 |
| (*Ulmus americana*) | 20.2 | 20.5 | 0.985 |
| Tobacco | 21.6 | 21.9 | 0.986 |
| (*Nicotiana tabacum*) | 11.4 | 11.5 | 0.991 |
| Tomato | 21.3 | 21.9 | 0.972 |
| (*Lycopersicon esculentum*) | 21.0 | 21.4 | 0.981 |

### Procedure for determination of respiratory ratios

The aggregate volume of the respiratory chamber *C*, separatory funnel *D* + *E*, and connecting tube to the zero mark on burette *A*, must first be determined as previously described. Germinating seeds serve most satisfactorily as the respiratory material in this apparatus, but other material can also be used. The manipulations will be described on the assumption that seeds are used. About 10 gm. of germinating seeds should be inclosed in a small wire mesh basket which should be so suspended from the rubber stopper *J* that it swings free from the bottom of the chamber by several

centimeters. The volume of the basket and seeds must be determined before they are introduced into the respiratory chamber and subtracted from the volume as determined above. After the apparatus is assembled, 30 cc. of 25 per cent. KOH solution should be introduced into the separatory funnel. The volume of the KOH solution must also be subtracted from the volume as determined above. The volume of the system, after subtraction of the volume of the seeds, basket, and KOH solution will be designated as volume V.

Each of the burettes should be about one-fifth full of water. After allowing the apparatus to stand for a few minutes, burette *B* (stopcock *H* open) should be raised or lowered until the water menisci stand at the same level in both burettes. The exact position of the water level in burette *A* should be recorded, and the volume of gas below the zero mark added to volume V. This will be designated as volume X. The temperature of the respiratory chamber and the barometric pressure must also be recorded at this time.

Stopcock *H* should now be closed and the apparatus allowed to stand for about two hours in the dark. At the end of this period the water in the two burettes should again be brought to the same level after opening stopcock *H*. Volume V plus the volume of gas below the zero mark on burette *A* at this reading will be designated as volume Y.

Stopcocks *E* and *I* should now be opened simultaneously, and the KOH solution allowed to flow into the bottom of the respiratory chamber. The KOH should not be allowed to come in contact with the seeds or the wire basket. About 15 minutes should be allowed for the absorption of any $CO_2$ gas in the system. The burettes must now be given a final adjustment until water stands at the same level in both of them. Volume V plus this final reading of the gas below the zero mark on burette *A* will be designated as volume Z. The temperature of the respiratory chamber and the barometric pressure must also be recorded at the end of the experiment.

All of the recorded gas volumes (X, Y, and Z) must be reduced to standard conditions and corrected for aqueous tension as in determinations of the photosynthetic ratio.

The significance of the several measured volumes in determinations of respiratory ratios is as follows:

$X = O_2 +$ all other gases (principally $N_2$) in the system, ($CO_2$ negligible)

$Y =$ unconsumed $O_2 +$ other gases $+ CO_2$ released in respiration

$Z =$ unconsumed $O_2 +$ other gases ($CO_2$ was absorbed by the KOH solution)

Hence:

$X - Z$ = volume of $O_2$ absorbed

$Y - Z$ = volume of $CO_2$ released

$\frac{(Y-Z)}{(X-Z)}$ = respiratory ratio

Representative results obtained in the measurement of the respiratory ratios of germinating seeds of various species by means of this apparatus are given in table II.

TABLE II

RESPIRATORY RATIOS

| SPECIES | $CO_2$ CONSUMED | $O_2$ RELEASED | RESPIRATORY RATIO, $\frac{CO_2}{O_2}$ |
|---|---|---|---|
| | *cc.* | *cc.* | |
| Wheat | 84.1 | 83.7 | 1.004 |
| (*Triticum aestivum*) | 61.1 | 60.3 | 1.013 |
| Soy bean | 4.4 | 6.8 | 0.646 |
| (*Glycine max*) | 9.4 | 14.6 | 0.649 |
| Sunflower | 5.7 | 6.5 | 0.876 |
| (*Helianthus annuus*) | 14.3 | 16.3 | 0.877 |
| Castor bean | 13.4 | 19.2 | 0.697 |
| (*Ricinus communis*) | 13.1 | 18.5 | 0.707 |

## Summary

The construction and manipulation of a relatively simple apparatus for the quantitative measurement of photosynthetic and respiratory ratios are described. Representative results selected from determinations of photosynthetic and respiratory ratios made with this equipment are presented in tabular form.

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# SOME RELATIONS BETWEEN LEAF AREA AND FRUIT SIZE IN TOMATOES[1]

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(WITH FOUR FIGURES)

## Introduction

Some years ago, when the senior writer was studying growth in tomato fruits, it was noticed that the later developed fruits in a cluster were always smaller than the first ones set (**1**); that in tomatoes the setting progresses from the base of the cluster and the apical flowers may produce no fruits. There seemed also to be a relation between the size, or perhaps age, of the plant at the time of setting of a fruit and the final size of that fruit. The fruits in the first cluster were invariably small and the setting was poor, only one or two fruits being produced. The size of the individual fruits as well as the number per cluster increased until the third or fourth cluster, when the maximum was reached. The later developed fruits were distinctly smaller. At the time it seemed reasonable to assume that these differences in size of fruits were due to the food supply of the plant. It seemed desirable therefore to find out to what extent the fruit size was dependent upon the available food supply. In order to do this a study was made of the relation between leaf area and fruit size in an experiment first conducted during the summer of 1932.

Before 1932, experiments had been conducted with apples, pears, grapes, peaches, and filberts (**2, 6, 7, 8, 9, 10, 12, 13, 14**), and a relation had been found to exist between the leaf area and fruit size. In 1911 HARRIS (**3**) found, in *Cladrastis*, that the heaviest seed in a pod was located next to the proximal (basal) end, and that there was a progressive decrease in the weight of the seeds toward the distal end. In beans, the same investigator (**4**) also found a correlation between the position of a seed in the pod and its weight, but in this plant the lightest seed was at the proximal end, with a progressive increase in weight toward the distal end, although the heaviest seed was usually not at the end but second or third from the distal end. Just which seed was heaviest depended upon the number of seeds in the pod; with few seeds in a pod the heaviest seed was at the end, but in pods with many seeds it was located several seeds from the distal end. HARRIS had observed in *Cladrastis* and in beans the same phenomenon that had been observed in the tomatoes, that the size varied with the position in the fruiting structure. He offered no explanation, however. SINNOTT (**11**), from his work on the bean and *Acer saccharum*, concluded that there was no correlation between

[1] Paper no. 554 from the Department of Botany of the University of Michigan.

the size of the body of a plant and the organs produced by it, such as leaves and fruits, but that these are correlated with the size of the growing point from which they were formed.

## Experimentation

In the summer of 1932, several hundred tomato plants were set out in the field. The plants were divided into four groups having one, two, three, or four leaves per plant. Each plant was permitted to produce fruits in only one cluster. Within each group of plants the clusters bore one, two, three, or four fruits. In this way there were obtained plants ranging from four leaves and four fruits to plants with one leaf and one fruit. The fruits were produced in either the second or third cluster.

The plants were not pruned to the desired number of leaves until the flowers had opened and at least one or two fruits had set. Consequently, the reduction in leaf area was operative only after the fruits had already been formed. Early fruit development was brought about by a plant with many leaves. In this way only one factor in the fruit growth was subjected to the experimental treatment, namely, its increase in size. According to SINNOTT the final fruit size had been determined by the time the leaves were removed and no effect of increasing or decreasing the leaf area per fruit could be obtained.

The experiments during this summer came to nought for two reasons. Crows, which had never done any damage before, destroyed many fruits, and thereby greatly reduced the number of plants that could be used. It was also found that when only a few leaves were left per plant the intense radiation of heat from the soil caused the leaves to curl, and, in some instances, to roll up. From this summer's work it became evident that the experiment must be conducted with a reduced light intensity, either in the field under shade or in the greenhouse.

In 1933 about 200 plants were grown in the greenhouse in 9-inch pots. Some plants were also placed out-of-doors for comparison but these were soon destroyed by the drought. The plants were pruned to one stem. Fruits were permitted to develop in the first, second, or third cluster and the leaves left were one, two, three, or four per plant. As in the preceding summer, they were not removed until some fruits had set. Only one, two, three, or four fruits were left on each plant. When only a few leaves were left on a plant there was a constant proliferation of new shoots, but these were removed as fast as they were formed. It would seem that considerable food material must have been used in the formation of these shoots. At the end of the experiment, the fruits were removed only as they matured. In some instances this reduced the number of fruits per leaf for some time, but by this time the fruits left were usually full grown, although not ripe, and

they probably did not derive any special benefit from the support of an increased leaf area. Blueprints were made of the leaves and the area determined with a planimeter. The area as given is for only one side of the leaf. When the final calculations had been made only 122 of the original 200 plants were left; the others had died during the summer, or had been discarded because of the loss of some of the fruits.

In analyzing the data, those plants that produced fruits in their first cluster have been omitted because there were too few of them. For the remaining plants, the efficiency (fruit-weight/leaf-area ratio) has been calculated and is represented in figure 1. For one plant with one leaf and

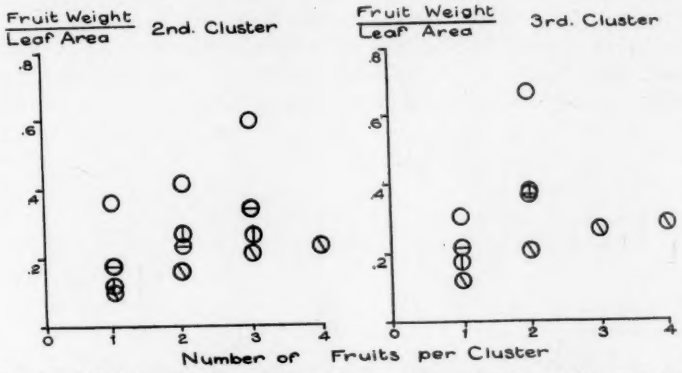


FIG. 1. Ordinate: Efficiency of the plant is represented by weight of fruits produced divided by leaf area. Abscissa: Number of fruits per cluster. Plain circles denote plants having only one leaf; circles with horizontal lines, plants with two leaves; circles with perpendicular lines, plants with three leaves; circles with slanting lines, plants with four leaves. Plants grown in summer of 1933.

three fruits in the third cluster, this value was 1.01, but as there was only one plant, it is not included in the graph. It will be noted that with one leaf the efficiency is the greatest, increasing as the number of fruits in the cluster becomes larger, but with an increase in the number of leaves the efficiency decreases. This is as one might expect, for, with a larger number of leaves some of the manufactured food probably is stored in the leaves and stems. No determination of dry material was made. The plants with several leaves were no larger than the plants with one leaf, and the plants with one leaf had the largest area per leaf. WEINBERGER (**12**) found that peaches produced by plants having fewer leaves had a lower sugar content and a lower percentage of dry material than fruits produced by plants having a larger number of leaves per fruit. There is no evidence for or against this in the tomatoes here discussed. However, from previous work

on the dry weight of tomatoes (1) it would seem that the percentage of dry weight was constant in this fruit. Judging from the color and taste, the fruits produced with one leaf per plant were normal. If the fruits and stems of plants with the larger number of leaves contain no more dry material than those of plants with only one leaf, it may be concluded that the plants with only one leaf are either more efficient, or that the rate of photosynthesis is greater. Can it be possible that photosynthesis is slowed down owing to the slow removal of the photosynthate, and that when the leaf area per fruit is small the removal of the photosynthate is more rapid? Since only one plant having one leaf produced four fruits, it seems probable that three fruits per leaf is the maximum production.

Because of the small number of plants of certain combinations that were carried through in 1933, the experiment was repeated in the summer of 1935. All of the fruits in 1935 were produced in the third cluster. The efficiency curves of these plants are shown in figure 2. These plants show

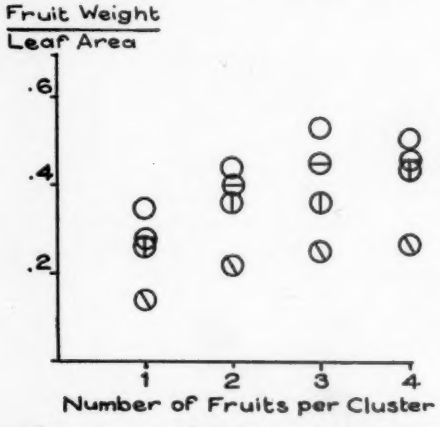


FIG. 2. Same as figure 1, except that plants were grown in summer of 1935.

even better the decrease in efficiency with increase in number of leaves than the plants experimented upon in 1933. Too detailed comparisons cannot, however, be made. For example, two leaves with two fruits are more efficient than one leaf with one fruit, while four leaves with four fruits are less efficient, and three leaves and three fruits are about the same as one leaf and one fruit.

Since the writers were interested not only in the efficiency of the leaves but also in the size which the fruits might attain, the data were examined for the purpose of studying the effect that the increase in leaf area may have had upon the size of the individual fruit. In figure 3, the weight of

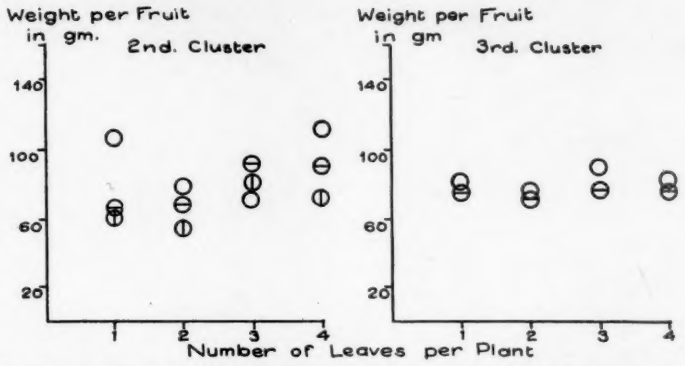


FIG. 3. Relation between individual fruit weight and number of leaves per fruit is shown. Plain circles denote one fruit per cluster; circles with horizontal lines, two fruits per cluster; circles with perpendicular lines, three fruits per cluster; circles with slanting lines, four fruits per cluster. Plants grown in summer of 1933.

the individual fruits is plotted against the number of leaves per plant. The figure shows no relation between fruit size and leaf area. This agrees with SINNOTT'S postulation that the fruit size is entirely determined by the number and not by the size of the cells. By the time the plants were pruned to the right number of leaves and number of fruits per cluster, the number of cells per fruit had already been established. Any increase in nutritional level could not, according to this theory, increase the size of the fruits.

During the 1935 season, the material was much more uniform in size, and the fruits were all produced in the third cluster. One hundred sixty-one plants were grown to the maturity of the fruits. Figure 4 shows a definite relation between individual fruit weight and number of leaves. With one fruit per plant the relation is almost linear up to three leaves per plant; but beyond this there is no further increase in fruit size with increase in leaf area. With one fruit per plant, when the number of leaves per plant was raised from one to three there was an increase of 40 per cent. in the weight of the fruit. With two fruits per plant the increase in fruit size continued even when four leaves per plant were left. The same is true for the plants with three and four fruits per plant. If it is true, as HOUGHTALING (5) states, that at the time of blossoming the ovary has the same number of cells which the fruit will have, it is obvious that the cells have reached their maximum size in plants having three leaves, and any further increase in the photosynthesizing area will have no effect upon fruit size.

The experiment demonstrates that by increasing the food producing area the fruit size can be increased considerably, although not in proportion

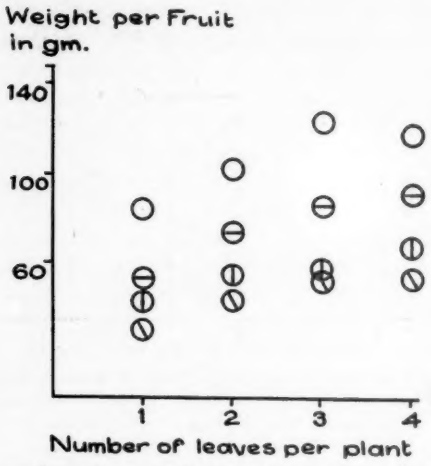


FIG. 4. Same as figure 3, except that plants were grown in summer of 1935.

to the increase in food material. This indicates that the fruit size is affected after setting as well as before. From the fact that the fruits did not increase in size in proportion to the increase in photosynthesizing area, it is obvious that factors other than nutrition at the time of enlargement influence the ultimate size of the fruit. How much could the fruits be increased in size by raising the nutritional level in the plant before the fruits have begun to develop? This question remains unanswered.

## Summary

1. The efficiency of the plant, as far as fruit production is concerned, is greatest when the leaf area per fruit is small.

2. By increasing the leaf area, the size of the fruit can be increased after the time of setting.

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# VARIATIONS IN THE CHIEF ASH CONSTITUENTS OF APPLES AFFECTED WITH BLOTCHY CORK

WALTER A. DELONG

In February, 1932, two samples of apples of the Fallawater variety, one badly affected with blotchy cork (locally known as "bitter-pit"), the other showing no symptoms of this disorder, were sent to this laboratory for analysis. These samples had been selected from the grading-table in a fruit warehouse and their tree origins were therefore unknown. They were known, however, to have been grown in the same orchard. The samples were analyzed for inorganic constituents and but one apparently significant difference was found, namely, in calcium content. Thus the affected fruit contained 0.052 gm. CaO per 1000 gm. fresh weight; the unaffected, 0.071 gm.

A favorable opportunity for checking this result on fruit collected directly from the trees presented itself in the autumn of 1934. At this time five samples of apples of the variety Stark were gathered as follows:

A. Fruit from a tree about 40 years old which had recently been severely headed back and was making the usual vigorous vegetative growth following this operation. It was bearing a full crop of fruit, which was estimated to be 5 to 10 per cent. affected with blotchy cork. The section of the orchard in which this tree was growing was inter-planted with corn for ensilage in 1934. All the apples collected showed many typical lesions and this sample was classified as showing very severe symptoms.

B. Fruit collected from a normally-pruned tree about 50 years of age located in an orchard about one-half mile from the preceding. This orchard was cultivated and cropped to oats and corn in 1934. The crop borne by the selected tree was heavy and it was estimated that about 3 per cent. of the fruit showed easily recognizable corky lesions at the time of collection. All the apples in this sample also showed numerous corky areas. The sample was classified as severely affected.

C. Fruit collected from a tree about 30 years old located in an orchard about midway between A and B. The sod mulch system of culture was followed in this orchard, the crop in 1934 was very light, the trees were not over-vegetative, and only a few fruits at the tops of certain trees showed blotchy cork symptoms. All the apples making up this sample exhibited corky lesions, however, and it was classed as slightly to moderately affected.

D. A composite sample made up as follows: (*a*) ten apples from the tree adjacent to the one from which sample A was taken, (*b*) ten apples from the same tree from which sample B was gathered, and (*c*) five apples each from two trees adjacent to the one from which sample C was taken.

None of the fruits of this sample C showed any visible corky lesions, although in the cases (*a*) and (*b*) the trees were certainly "cork-susceptible" in 1934, and possibly also in case (*c*).

E. Fruit from trees grown at the Dominion Experimental Farm at Kentville, Nova Scotia. These trees have been under continuous observation since they began to bear and have never been found to produce fruit affected with any of the so-called physiological disorders of the cork type. A system of clean cultivation followed by a cover-crop of vetch is the practice in this orchard and the trees are 25 years old.

Samples A–D inclusive were gathered September 22, 1934, at Windsor Forks, Nova Scotia. Sample E was collected September 26. The soils in the various orchards are of similar type, ranging from a sandy loam at Kentville to a gravelly soil in the case of orchard C at Windsor Forks.

After collection, the samples, which consisted of 30 apples each, were at once taken to the laboratory where they were pared and sliced for drying. The parings and the flesh of the fruit, the latter minus the seeds and stems, were dried, ashed, and analyzed separately. The methods of analysis followed were those of the A. O. A. C. except that the magnesium, after separation from iron, calcium, etc., was determined by the hydroxy-quinoline method, and the potassium by the butyl alcohol-perchlorate method. The analytical results are reported in table I.

TABLE I

ASH ANALYSES OF STARK APPLES, A, B, C, AFFECTED WITH BLOTCHY CORK, D AND E UNAFFECTED

| SAMPLE | ASH IN FRESH WEIGHT | PERCENTAGE IN THE ASH | | | | AVERAGE FRESH WEIGHT PER APPLE |
|---|---|---|---|---|---|---|
| | | $P_2O_5$ | CaO | MgO | $K_2O$ | |
| | % | % | % | % | % | *gm.* |
| | ANALYTICAL RESULTS ON PARINGS | | | | | |
| A | 0.528 | 10.2 | 1.27 | 4.32 | 56.1 | 124.1 |
| B | 0.550 | 10.5 | 1.56 | 4.53 | 55.4 | 119.7 |
| C | 0.627 | 12.6 | 1.67 | 4.67 | 55.8 | 132.7 |
| D | 0.478 | 11.1 | 2.41 | 4.54 | 55.5 | 124.9 |
| E | 0.476 | 10.2 | 3.57 | 4.61 | 53.2 | 120.2 |
| | ANALYTICAL RESULTS ON FLESH | | | | | |
| A | 0.243 | 9.5 | 1.67 | 3.33 | 56.1 | ..... |
| B | 0.256 | 10.8 | 1.80 | 3.58 | 55.1 | ..... |
| C | 0.264 | 11.1 | 1.76 | 3.43 | 56.2 | ..... |
| D | 0.238 | 10.1 | 2.32 | 3.54 | 56.2 | ..... |
| E | 0.235 | 11.6 | 2.99 | 3.63 | 54.7 | ..... |

The results obtained in table I are seen to confirm those found in 1932, the most striking deviation in inorganic composition of affected as compared with unaffected fruit (sample D) and with fruit from non-susceptible trees (sample E), and probably the only significant one, being found in the calcium content. It is true that sample C shows a somewhat high ash content and content of phosphorus in the ash. This, however, is probably to be attributed to the fact that these trees were bearing a light crop in 1934 (**2**), rather than to any specific effect related to the incidence of blotchy cork.

In the case of the calcium content, the percentage values of table I show a striking diminution of this element as the severity of attack of the disorder increases. From the physiological standpoint a more correct picture of the situation with respect to this element is doubtless given by calculating the amount present per unit of fresh weight. When this is done and the results are expressed as percentages of the quantity of calcium present per unit of fresh weight in sample E (*i.e.*, fruit from non-susceptible trees), the results shown in table II are obtained.

TABLE II

RELATIVE AMOUNTS OF CALCIUM PER UNIT OF FRESH WEIGHT

| SAMPLE | PARINGS | FLESH |
|---|---|---|
| A | 40 | 59 |
| B | 51 | 67 |
| C | 62 | 67 |
| D | 68 | 79 |
| E | 100 | 100 |

The cause of this apparent calcium deficit in fruit affected with blotchy cork is uncertain. It has been shown (**5**), however, that of the total amount of this element in the non-woody tissues of the apple tree (the leaves and fruit), some 90 per cent. is found in the leaves. It is also well established that blotchy cork commonly occurs on what may be classed as over-vegetative trees. Further, unpublished data obtained in this laboratory (**3**) indicate that calcium is being absorbed most rapidly by the growing fruit coincidently with the most rapid production of new shoots and leaves by the tree. It is therefore suggested that a low calcium content in apples affected with this disorder may arise as a result of competition between leafy shoot and fruit tissues for this element during these early stages of growth. Possibly this apparent calcium deficit may play a part in the origin of the lesions typical of this disorder. In this connection it is also interesting to note that boron is reported (**4**) to be associated in some way with the calcium nutrition of plants, and that ATKINSON has recently stated (**1**) that he

has obtained amelioration of cork symptoms on fruit following treatment of apple trees with boric acid.

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# INFLUENCE OF IRRIGATION ON THE DEVELOPMENT OF THE ROOT SYSTEM OF SPRING WHEAT PLANTS

N. A. MAXIMOV AND A. S. KRUŽILIN

## Introduction

The purpose of this work was to follow the development of the root system of spring wheat plants under irrigation given at different times and in different quantities. The rate and character of root development are very important factors in the determination of irrigation methods for wheat.

This work was carried out in the summer of 1933 in the Volga district at the Valuika Experiment Station on soils of the solonetz-chestnut type. Irrigation was practiced by means of flooding. Meteorological conditions of this summer were characterized by a higher than normal rainfall; that is, a 163-mm. instead of 126-mm. precipation during the growing season.

Observations of root development were made by two methods: the trench method of WEAVER, and the weighing method of KACHINSKY (**2**) (washing out and weighing of the roots obtained from soil columns at different soil horizons). Records were taken at three periods: at the time of tillering, during the time of the development of heads, and at full maturity.

## Investigation

Two series of plants were under observation: Series I at a normal time of sowing (April 26) and without any soil irrigation before sowing; series II at the time of a late sowing (May 30) and with an irrigation (May 23) before sowing. For series I soft wheat, *Erythrospermum* 0841, was used; for series II hard wheat, *Melanopus* 069. Thus the two groups of wheats were different and the results are therefore not strictly comparable.

The investigation was carried out with 1, 2, and 3 irrigations. About 1200 cubic meters of water were applied at each irrigation. The total quantity of water given to the plants irrigated once was 1200 cubic meters; to those irrigated twice, 2400 cu. m.; and to those irrigated three times, 3600 cu. m.

Comparing the plants in the groups that were irrigated with those not irrigated it is evident that: the chief root mass of the irrigated plants is situated in the higher soil horizons, up to 40 cm. below the surface, where 80 per cent. (by weight) of all the roots were found; the root systems of plants grown without irrigation had a tendency to develop deeper (table I).

In the root systems of plants grown with irrigation there was a spreading of the nodal roots in the surface layer of soil. Their number increased after each irrigation. The nearer to the surface the chief mass of roots of the

irrigated plants was, the better they were able to utilize the upper soil horizon, which is rich in nutrient substances.

TABLE I

DISTRIBUTION OF WHEAT ROOTS IN THREE SOIL HORIZONS AT TIME OF MATURITY OF THE PLANTS. RESULTS IN GRAMS PER PLANT

| SOIL HORIZONS | 0–20 CM. | 20–40 CM. | 40–60 CM. |
|---|---|---|---|
| SERIES I | *gm.* | *gm.* | *gm.* |
| Without irrigation | 3.32 | 1.73 | 1.91 |
| 1 irrigation, on June 1 | 5.98 | 3.04 | 1.77 |
| 1 irrigation, on June 19 | 5.78 | 2.83 | 1.98 |
| 2 irrigations, on June 1 and 29 | 7.56 | 3.26 | 1.91 |
| 2 irrigations, on June 1 and July 7 | 7.42 | 3.03 | 1.85 |
| 3 irrigations, on June 1 and 19, and July 7 | 7.65 | 3.37 | 2.07 |
| SERIES II | | | |
| Without irrigation | 5.16 | 1.83 | 1.84 |
| 1 irrigation, on June 30 | 8.91 | 2.58 | 2.03 |
| 1 irrigation, on July 20 | 6.03 | 2.20 | 1.92 |
| 3 irrigations, on June 30, and July 15 and 29 | 10.73 | 2.12 | 1.64 |

A comparison of the various groups indicates a close connection between the time of irrigation and the development of the nodal roots.

Table II shows root development during certain phases of plant growth in the various groups of series I of our experiments (without use of water before sowing). In the case of plants without irrigation, only 26 per cent. of the nodal roots developed after the tillering stage; in the irrigated plants, each use of water brought an intensive root growth. The plants belonging to the second group (with 1 irrigation, on June 19), at the time when the heads were forming, gave new nodal roots from the tillering shoots only after the use of water. Consequently the tillering shoots developed 16 to 20 days later than the main stems. This resulted in a lower yield of grain and of total dry matter.

If water is made available somewhat earlier, at the end of tillering and beginning of stem formation of the plants, then the chief growth of roots takes place at the most critical period, that is, during the time of intensive development of the above-ground parts. Nearly all of the roots are formed during the 3-week period from the time of tillering to the time of head formation. It is therefore highly desirable to apply water for the first time before this important period.

The branching of roots of the irrigated plants was more or less uniform in all soil horizons, but those without irrigation branched more at a depth of 40–60 cm., hence this horizon had a second maximum of root accumula-

## TABLE II

INCREASE OF ROOTS AT CERTAIN STAGES OF DEVELOPMENT OF PLANTS OF SERIES I. RESULTS IN PERCENTAGE OF TOTAL WEIGHT OF ROOTS

| TIME OF SAMPLING | TILLERING STAGE MAY 30 | HEADS FORMING JUNE 17 | MATURITY JULY 15 |
|---|---|---|---|
| | % | % | % |
| Without irrigation | 74 | 21 | 5 |
| 1 irrigation, on June 1 | 48 | 49 | 3 |
| 1 irrigation, on June 19 | 48 | 14 | 38 |
| 2 irrigations, on June 1 and 24 | 40 | 35 | 25 |
| 2 irrigations, on June 1 and July 7 | 42 | 36 | 22 |
| 3 irrigations, on June 1 and 19, and July 7 | 40 | 35 | 25 |

tion. CHIŽOV (**1**) and ORLOVSKY (**3**) found this second maximum of roots during years of drought. The writers did not notice it under irrigation as did ORLOVSKY. Perhaps this was the result of "softening" of the soil by the irrigation water.

If the soil is irrigated early enough, the nodal roots penetrate more than 1 m. Throughout the period of growth new nodal roots appear after each irrigation, and, with sufficient application of water, the total quantity of such roots is two to three times as great as without irrigation.

Plants of the group receiving three irrigations had but a slight increase in root development in comparison to those with two irrigations. The difference in the development of the above-ground tissues was also insignificant. This suggests that if 1200 cu. m. of water are used, the third irrigation is unnecessary.

A feature characterizing series II of the experiments, with water used before sowing, was a still greater tendency for the surface spreading of the root system, which could be seen at the beginning of the tillering stage. But since the sowing was done one week after the irrigation, we did not obtain the expected intensive development of nodal roots. At the time of tillering, the branching nodes were in a perfectly dry soil and the nodal roots, 2 to 3 in number, were only 5 to 8 cm. long.

Owing to irrigation and the rainfall that followed it, important changes were brought about. Samples taken during the tillering stage showed clearly the surface situation of the roots, and, at the time of maturity this was still more pronounced. The percentage of roots and their total amount (by weight) was considerably higher in the plowed horizon of series II than of series I (without irrigation before sowing). Late irrigation also decreased the number and development of nodal roots of series II and was very in-

jurious to normal tillering. Hence, in the case of irrigation before sowing, the first use of water after sowing must not be later than during the period when stalking is about to begin.

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## BRIEF PAPERS

### CARBOHYDRATES IN *IRIDEAE LAMINARIOIDES* (RHODOPHYCEAE)

The carbohydrates in the higher plants have been extensively, although by no means completely, studied. Comparatively little work has been done with regard to the chemical constituents of the algae.

The carbohydrates present in the algae are of a different nature from those found in higher plants. Those commonly occurring in the latter are glucose, sucrose, fructose, starch, cellulose, and hemicelluloses. In the algae, soluble sugars such as glucose, sucrose, and fructose are either completely absent, or where they appear, they do so only in comparatively small amounts. Instead, sugar alcohols, mannitol, dulcitol, and sorbitol seem to predominate. Of the polysaccharides, such compounds as algin, laminarin, fucosan, galactan, and floridian starch have been reported present.

In view of the different nature of the carbohydrates found in the lower plants, their carbohydrate metabolism apparently takes a different course from that in the higher plants.

The various polysaccharides reported in the algae have been inadequately studied, and apparently there is as yet no precise knowledge of their chemical nature and composition. They are described in such vague terms as "laminarin, a levo-rotatory substance which, on hydrolysis, gives glucose" (**6**). Another polysaccharide fucoidin, reported by BIRD and HAAS (**1**), is described as "a calcium salt of a sulfuric acid ester of a non-reducing polymerized uronic acid complex combined with a methylpentosan." Other polysaccharides found in the algae are described in similar vague terms.

The writer has studied the carbohydrates occurring in a red alga, *Irideae laminarioides*, and has isolated a galactan which proved to be a sodium salt of a sulphuric acid ester of galactan (**3, 4**). The sulphuric acid group of this compound could be split off and a polysaccharide was thus obtained which, in the chemical sense, is a pure galactan. This is apparently the first time that a homogeneous galactan, consisting entirely of galactose units, was obtained from a plant.

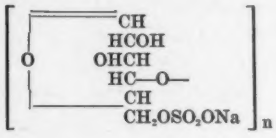
Plants of *Irideae laminarioides* were collected at four different times of the year, extracted with alcohol, and analyzed for soluble sugars. No free reducing or total sugars were found. On concentrating the alcoholic extract, however, a crystalline substance was obtained, which was identified as the sugar alcohol, dulcitol (**2**).

The plants, after being extracted with alcohol and then benzene, until free of chlorophyll, were ground and dried. This dry material, practically all soluble in water, was heated with water, filtered, the solution concen-

trated and then poured into 95 per cent. alcohol. A precipitate in the form of white flocculent threads was obtained. After several reprecipitations and drying, a snow-white substance was obtained which did not reduce Fehling's solution and was free of nitrogen. Its specific rotation $[\alpha]_D$ was +69.2. It was a neutral substance; a solution in water had a pH of 6.8. This new carbohydrate consisted of galactose units and contained a sulphur group.

The carbohydrate was studied by chemical methods such as hydrolysis, acetylation, methylation, oxidation, etc., and its derivatives, diacetylsodium sulphuric acid ester of galactan, trimethylgalactan, trimethyl-galactose, methyl-arabodimethyoxyglutarate, dimethoxyhydroxy glutaric acid and others were examined.

These studies showed this carbohydrate to be a sodium sulphuric acid ester of galactan. A structural formula as shown was proposed for this substance in which $n$ seems to be approximately 6.




The sulphuric acid group in this compound could be removed by hydrolysis with an acid or base, and a galactan composed entirely of galactose units was obtained.

When the sodium sulphuric acid ester of galactan was subjected to electrodialysis, the sodium was removed and the substance initially neutral became acid, pH of about 3. The resulting substance was sulphuric acid ester of galactan. The neutrality of the compound, the shape of its titration curve, and its specific conductance indicate that the sodium sulphuric acid ester of galactan is a salt of a strong acid.

The leaves of the *Irideae laminarioides* when digested with hot water gave the characteristic blue starch test with iodine. The leaves were subjected to the influence of diastase and the starch was definitely identified by preparing a maltosazone from the products of hydrolysis. Contrary to the theory held by investigators (5) that starch is never found in red algae, it was found to be present in *Irideae laminarioides*. A small amount of pentosan material also was found.

The plant does not contain any cellulose, and since the content of galactan is about 40 per cent. of its dry weight and constitutes the main bulk of the plant, it appears that this substance plays the same part in building up its cell wall as does that of cellulose in the higher plants.

The sulphur content in the plant is high (about 6 per cent.) and exists

entirely in organic combination as an ethereal sulphate. The rôle of this ethereal sulphate in the plant is not known.

The anomalous carbohydrate metabolism of *Irideae laminarioides* is difficult to explain, in view of the fact that no free sugars were found to be present, but instead a sugar alcohol, dulcitol. Perhaps sugars are formed in this plant, but their rate of formation is conditioned by the same rate of utilization and, therefore, they are not detectable; this is a question which requires further study. The formation of dulcitol and its rôle in metabolism of the plant are also obscure. It is possible to assume that galactose is formed as the first product of photosynthesis and then immediately transformed partly into galactan and partly reduced to dulcitol. Since starch is also found to be present in the plant, glucose might perhaps be the first product of photosynthesis and there is a mechanism in operation which permits the transformation of glucose into galactose.

These, however, are mere assumptions and illustrate the great complexity of carbohydrate metabolism in the plant.—W. Z. Hassid, *University of California, Berkeley, California.*

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## COLORIMETRIC DETERMINATION OF NARINGIN

The writers have made numerous quantitative determinations of naringin in grapefruit rinds by the method which depends upon the reducing power of the hydrolyzed glucoside. The method was an adaptation of that used for phloridzin (**1, 2, 3**). When this method was employed in conjunction with regular sugar analyses it was satisfactory, but when, as in the present

case, it was desired to follow the changes in naringin content alone it was necessary to develop a more simple and rapid method.

In the search for a method it was found that the vinaceous red color resulting from the interaction of naringin and ferric chloride could be used for quantitative colorimetric determinations. The new colorimetric method is rapid and apparently more accurate than that previously employed. The naringin content of one or more samples of fresh tissue can be determined within less than one hour. Naringin may, if necessary, be determined from samples of fresh tissue as small as 0.1 gm. The most serious objection to the method lies in the fact that the color, after formation, continues slowly to deepen. To overcome this behavior it is necessary to develop uniform manipulation, especially in the timing. Furthermore, the tissue extracts must be relatively free of pigment to allow satisfactory comparison with the pure standards. The flavedo, but not the albedo, portion of grapefruit rind, offers some difficulty in this respect.

The colorimeter used by the writers is the Duboscq type with 50 mm. tubes and translucent scales marked with 1 mm. graduations. In place of the usual mirror mount there is installed a daylight colorimetric lamp.

The standard solution is made from a 0.2 per cent. naringin[1] stock solution in 95 per cent. ethanol. One ml. of this solution diluted to 25 ml. with water becomes the standard solution. Immediately before using, one drop of a 50 per cent. ferric chloride solution is added to the standard solution which is then transferred to the right hand colorimetric tube, and the vernier set at 50 mm.

The general procedure for the preparation of grapefruit rind extract is as follows: Separate a quantity of albedo from the flavedo and grind it in a food chopper through the nut-butter attachment. Mix this ground tissue and weight out 10 gm. Transfer the 10-gm. sample to an 800-ml. beaker and cover with 500 ml. of hot water. Boil for 10 minutes and strain through cheese cloth into a 500-ml. volumetric flask. Cool the solution and dilute to 500 ml. by adding water. This extract may be used in the left hand colorimetric tube with or without further dilution, depending upon the approximate percentage of naringin in the tissue. The amount of dilution may be determined from the following general statements: (a) For tissue containing less than 1 per cent. of naringin by fresh weight no further dilution is necessary. Immediately before transferring the material to the colorimetric tube add 1 drop of 50 per cent. ferric chloride solution for each 25 ml. of extract. (b) For tissue containing 1 to 2.5 per cent. of naringin dilute 10 ml. of the extract to 25 ml. with water and add one drop of 50 per cent. ferric chloride solution. (c) For tissue containing 2 to 4 per cent. of naringin dilute 10 ml.

[1] Naringin can be purchased from the California Fruit Growers Exchange, Ontario, California. This material should be dissolved in alcohol, filtered and recrystallized before using.

of the extract to 50 ml. with water and add two drops of 50 per cent. ferric chloride solution.

The calculation of naringin from the colorimeter scale readings is simple. The standard solution contains 8 mg. of naringin per 100 ml. The extract is always diluted so that it contains somewhat more naringin than the standard solution, thus all readings for the unknown are less than 50 mm. Assume that for a certain dilution of rind extract the reading is 40 mm. Then the amount of naringin in such a dilution will be $\frac{50}{40}$ of 8 mg., or 10 mg. per 100 ml. The writers have made a curve of reference for each dilution of extract so that any scale reading can be translated directly and quickly into percentage of naringin in the original fresh tissue. For percentage of naringin on a dry weight basis reference must, of course, be made to the results from corresponding samples taken for dry weight determinations.

As an aid in avoiding the gradual deepening of the color after formation, the standard solution in the colorimeter tube is renewed frequently and easily by making up a number of 25-ml. portions of the standard solution before beginning a series of determinations. Each of these portions is ready for the colorimeter except for the addition of one drop of 50 per cent. ferric chloride solution which should be added, in each case, just before the material is placed in the colorimeter.

Temperature, within a wide range, has practically no effect upon the quality and intensity of the initial color reaction, but it has a noticeable effect upon the rate of deepening of that color afterward. It has been observed also that the 0.2 per cent. naringin stock solution after standing at room temperature undergoes a slow change which is equivalent to about 0.005 per cent. naringin per day if calculated on the basis of the fresh weight of grapefruit rind. These facts suggest that the colorimetric procedure should be carried out under uniform room temperature, and that the naringin stock solution should be made up in quantities which can be used within one or two days.—E. M. Harvey and G. L. Rygg, *U. S. Department of Agriculture, Division of Fruit and Vegetable Crops and Diseases, Pomona, California.*

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## NOTES

**Spring and Summer Meetings.**—Several meetings of the American Society of Plant Physiologists and its sections are in immediate prospect. The New England section holds its annual meeting at the Connecticut State College, Storrs, Connecticut, May 8–9. The principal feature of the meeting is a symposium on light and light measurements. The subject was chosen at the New Hampshire meeting last year. The desirability of projecting symposia long in advance of the meeting dates is evident, and the New England section is to be congratulated on its far-sighted policy. It is hoped that a brief account of this meeting may be presented in the July number of PLANT PHYSIOLOGY.

The Western section will initiate its work by a meeting at Seattle in June, when it meets with the Pacific Division of the A. A. A. S. The sessions will be held during the week of June 15–20, 1936. Although Seattle is far from the center of the regional territory, it is hoped that there will be a good attendance, and an attractive program.

The summer meeting of the Society will be held during the same week in Rochester, New York. The program committee appointed by President MURNEEK consists of the following members: H. C. THOMPSON, Cornell, chairman; C. G. DEUBER, Yale University; R. B. WITHROW, Purdue University; and Z. I. KERTESZ, New York Agricultural Experiment Station, Geneva. Information regarding details of the meeting will be sent to members in due time. Suggestions for the meetings are welcomed by those who are responsible for the development of the programs. Communications may be addressed to the secretary, or to the officers of the sections.

**International Address List.**—The international address list was originated in the belief that it would help to draw plant physiologists of the entire world into friendly and cooperative relations with one another. The list has now been issued in three editions at about five-year intervals. Although much care is exercised in its production, it is not possible to avoid some errors in the addresses, omission of names, and similar inaccuracies. Moreover, such a list is no sooner issued than it begins to become obsolete because of changes in address of individuals listed. The editor of this bulletin, Dr. R. B. HARVEY, University of Minnesota Farm, St. Paul, will appreciate any corrections that may be offered, and information concerning changes of address as soon as they occur. It is hoped that the address list may be useful to plant physiologists everywhere, and that it may facilitate exchanges of publications, and correspondence between investigators in different countries who are interested in similar problems.

**Went Portrait.**—The editors of PLANT PHYSIOLOGY are much pleased

to be able, through the courtesy of G. J. PEIRCE, to present as a frontispiece to this issue, a reproduction of the H. J. HAVERMAN pastel of WENT from the Collection of Portraits of Eminent Naturalists at Stanford University. The original pastel was drawn from life in 1921. Copies of this portrait will be made and kept with those which are now available for purchase in sets. If copies are desired, they may be obtained from the editor-in-chief of PLANT PHYSIOLOGY.

**Bibliofilm Service.**—Science Service has established a bibliofilm service in the Library of the U. S. Department of Agriculture, Washington, D. C. The cost is very small. A page of copied material, microfilm, is quoted at one cent per page, photoprints at five cents each. There is a service charge of ten cents for each item copied, and a minimum of twenty-five cents on acceptable orders. Service charge for special delivery air mail transmission is twenty cents.

This service will also be available for publication of tabulated data, diagrams, photographs, etc., which exceed the limits allowed by scientific journals. It is not at present the intention of PLANT PHYSIOLOGY to make use of this method of publishing excess pages, data, or cuts. The attention of authors is called to this new service, however, in the hope that those who find current limitations of the journals too restrictive may place their data on record in this inexpensive way. If additional information is wanted, inquiries may be addressed to Mr. WATSON DAVIS, Director of Science Service, 2101 Constitution Ave., Washington, D. C.

**Russian Language Courses.**—Plant physiologists have many incentives to acquire a reading knowledge of the Russian language. It is interesting to note that opportunities are again open for intensive short courses in Russian. At the University of California, the summer session is offering ten weeks' intensive study for mature students and scholars. The first course is a grammar, reading, composition, elementary conversation course, while the advanced work is open only to those who have had a year of Russian or its equivalent. The first course presupposes no previous knowledge of Russian, but knowledge of some other language than English would be helpful. Students are expected to spend five and one-half hours daily, five days a week. The tuition for either course is $100, and the University fee. The session begins June 22, and closes August 29.

The Harvard summer school is also offering elementary Russian this summer, the term running from July 6 to August 15. Tuition in this case is $60, and the enrollment is limited to 15 students. The ability to read Russian contributions to plant physiology is an asset that any American plant physiologist might well covet. With these opportunities for study of Russian open to students during the summer, it would not be difficult

to acquire a reading knowledge that would enable one to go directly to the original Russian contributions in our field.

**Protoplasm.**—The McGraw-Hill Book Company has published an interesting and valuable monograph entitled Protoplasm. The author is Dr. WILLIAM SEIFRIZ, Professor of Botany at the University of Pennsylvania. The book contains 584 pages, and 179 illustrations. The story of protoplasm is told in 27 chapters, which cover the ground admirably for the student. The early chapters deal with the general properties of protoplasm and methods of studying it, model making, micrurgy, tissue culture. Then follows numerous chapters dealing with the colloidal state, emulsions, sols and gels, surface tension, adsorption, osmosis, imbibition, viscosity, elasticity. The structure of protoplasm and organic colloidal matter is then considered, and such problems as acidity, electrophysiology, electrokinetics, radiant energy, rôle of water, salts, carbohydrates, fats, proteins, and regulatory substances. The final chapter deals with the origin of living matter. It is a mine of information, a very useful summary of our knowledge of protoplasm at the present time. The price quoted by the publishers is $6.00 per copy.

**Harris, Botanist and Biometrician.**—A biographical memorial volume commemorative of the life and work of Dr. J. ARTHUR HARRIS, who was at the time of his death on April 24, 1930, Professor of Botany and Biometry and head of the Department of Botany at the University of Minnesota, has been published by the University of Minnesota Press. The work was edited by three of Dr. HARRIS's colleagues, C. O. ROSENDAHL, R. A. GORTNER, and G. O. BURR. It contains a brief chronology of his life; an appreciation of HARRIS the Man, by GORTNER; HARRIS's presidential address before the American Society of Naturalists at Philadelphia in 1926; an appreciation of HARRIS the Botanist, by ROSENDAHL; and of HARRIS the Biometrician, by ALAN E. TRELOAR. In addition there are six papers by HARRIS and his coworkers, and a complete bibliography of the published works of HARRIS, arranged in chronological order. One of the most attractive features of the book is the fine portrait of HARRIS at the age of 44, which forms the frontispiece. There are eight additional photographs reminiscent of HARRIS's trip to Hawaii in 1924, and of his field work in Utah in 1920. The book is attractively bound, and is a very satisfactory memorial to one of the most indefatigable workers, one of the most vigorous and lovable personalities among American botanists. It is offered at $2.50 per copy by the University of Minnesota Press, Minneapolis, and will be prized by every possessor.

**Bibliography of Low Temperature Relations.**—A revised edition of Dr. R. B. HARVEY's Annotated Bibliography of the Low Temperature Rela-

tions of Plants has been issued by the Burgess Publishing Co., Minneapolis, Minnesota. The first edition met wide acclaim, and those who failed to secure a copy of the first edition can now obtain the revised edition of 1936 for the same price, $4.00 per copy. The revision contains about sixteen pages of additional references. These are included in an appendix following the 3412 references of the first edition. This work is one of the most important compilations of literature citations that has been made available to plant physiologists. It is indispensable to anyone interested in low temperature relations from any standpoint.

**Shelterbelt Planting.**—The proposed Shelterbelt Zone has its best foot foremost in an attractive book entitled Possibilities of Shelterbelt Planting in the Plains Region, which was prepared under the direction of the Lake States Forest Experiment Station, and published by the U. S. Forest Service. The problems and the possibilities of solving them are considered in 14 sections. The problem as conceived by the Forest Service is set forth briefly by F. A. Silcox, chief of the Service. Preliminary findings and recommendation are outlined by R. Zon, and then follows sections dealing with geographic problems, the proposed establishment and management of tree plantations, land acquisition, prospective effects of tree planting, a survey of past plantings and a review of early tree planting activities in the Plains region, shelterbelt experiences of other lands, economic and social aspect of agriculture in the region, climatic characteristics of the Plains, soil and forest relationships of the shelterbelt zone, native vegetation of the region, and groundwater conditions of the shelterbelt zone. It is profusely illustrated with more than a hundred figures, maps, etc. The discussions are very illuminating, and aside from the prohibitive cost of the shelterbelt, the difficulties in establishing trees generally over such a region, and its problematical effects upon the natural climate and soils of the region, the project is given a very impressive showing. Anyone who reads it carefully will have a much better understanding of the natural forces with which man must cope in his efforts to improve the climate and soil conditions of a vast region. The Forest Service has rendered a distinct service in preparing this well rounded symposium on the scientific aspects of this great problem.